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Canine (*Canis familiaris*) Scent Detection of Invasive Brown Bullhead Catfish (*Ameiurus nebulosus*) in Water Samples, and the Effects of Sample Preservation

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Abstract

Brown bullhead catfish (*Ameiurus nebulosus*) are an invasive freshwater fish widely recognised as a threat to New Zealand's aquatic ecosystems. Early detection of new incursions increases the chances of eradication or control, but current survey methods such as netting and electrofishing are expensive and time consuming when fish are at low abundances. Domestic dogs (*Canis familiaris*) have previously demonstrated the ability to detect the presence of catfish in laboratory water samples, while discriminating against non-target species. The aim of this research was to investigate whether scent detection dogs could plausibly be employed in aquatic biosecurity programmes. The first experiment used a probe design to determine the scent-detection limits for catfish biomass under laboratory conditions. Water samples taken from tanks containing a standard catfish biomass concentration of 15.5 g/L (equivalent to 38,700 kg/ha) were assessed by the dogs and progressively diluted following the successful attainment of identification and discrimination criteria. All three participating dogs were able to detect the presence of catfish at biomass concentrations ≤12.6 kg/ha, with one dog achieving 4.6 kg/ha. Concurrently, the dogs were also able to successfully discriminate against a goldfish (*Carassius auratus*) distractor scent indicating that they are able to distinguish specific species of fish in the environment. The effects of sample preservation on dogs' ability to identify and discriminate target scents have rarely been investigated. In practice, field samples from the environment would likely necessitate the preservation of samples prior to assessment by dogs. A second study used a repeated-measures reversal design to investigate dogs' abilities to detect catfish at a standard biomass concentration of 310 kg/ha which had been refrigerated or frozen for up to 1-week. No statistically significant difference in hit rates between unpreserved (baseline 1: 93%, baseline 2: 98%), refrigerated (97%), and frozen (96%) samples was apparent; ($F(3, 6) = 2.061502$, $p = 0.2069$). These results indicate that dogs are capable of detecting and discriminating the scent of catfish at biomass concentrations typical of many freshwater ecosystems in the Waikato region. Further, limited periods of freezing or refrigeration appear to have no significant effect on the scent-detection abilities of dogs. Future research needs to be conducted with field samples from lakes and could explore the effects of preservation on low biomass concentrations. The results from this study are promising for the potential future employment of scent detection dogs in aquatic biosecurity monitoring.

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Table of Contents

Abstract i
Acknowledgements ii
Table of Contents iii
List of Figures vii
List of Tables ix
Chapter 1 Introduction 1
1.1 Freshwater Ecosystems 1
1.1.1 New Zealand's freshwater ecosystems 1
1.1.2 Brown bullhead catfish 2
1.1.3 Current aquatic species detection methods 3
1.2 Scent Detection Dogs 6
1.2.1 Conservation dogs in New Zealand 7
1.2.2 Scent detection dogs and water 7
1.2.3 Dogs in aquatic conservation and biosecurity 8
1.2.4 Preservation of odours samples 9
1.2.5 Limitations of scent detection dogs 10
1.2.6 Resolutions 11
1.3 Project Aim and Thesis Structure 11
Chapter 2 Biomass Limits for Scent-detection of Catfish 13
2.1 Introduction 13
2.2 Methods 14
2.2.1 Animal ethics statement 14
2.2.2 Recruitment and selection of dogs 14
2.2.3 Fish collection and general maintenance 15
2.2.4 Experiment apparatus 15
2.2.5 Dog training 18
2.2.6 Training procedure 18

2.2.7 Experiment design 20
2.2.8 Experimental procedure 20
2.2.9 Sample collection and preparation 22
2.2.10 Cleaning 25
2.2.11 Statistical analysis 25
2.3 Results 28
2.3.1 Accuracy 29
2.3.2 Final sessions 34
2.3.3 Probe accuracy in high accuracy sessions 34
2.3.4 d’ calculations 36
2.3.5 Accuracy relative to chance 36
2.3.6 Introduction of Possyum 39
2.4 Discussion 40
2.4.1 Biological relevance 40
2.4.2 Session numbers 40
2.4.3 Hit rates and correct rejection rates 41
2.4.4 Distinguishing signals from noise 42
2.4.5 Accuracy relative to chance 43
2.4.6 Probe accuracy in high accuracy sessions 43
2.4.7 Change in reinforcer 44
2.4.8 Conclusions 44
Chapter 3 Preservation Experiment 45
3.1 Introduction 45
3.2 Methods 46
3.2.1 Animal ethics statement 46
3.2.2 Recruitment and selection of dogs 46
3.2.3 Preservation experiment design 46
3.2.4 Experimental procedure 47
3.2.5 Water sampling and sample preparation 47

3.2.6 Collection and preparation of baseline samples 48
3.2.7 Collection and preparation of refrigerated samples 48
3.2.8 Collection and preparation of frozen samples 48
3.2.9 Cleaning 49
3.2.10 Statistical analysis 49
3.3 Results 50
3.3.1 Daily means 50
3.3.2 Summary accuracy results 52
3.3.3 d’ calculations and ANOVA 54
3.3.4 Accuracy relative to chance 54
3.4 Discussion 57
3.4.1 Refrigeration phase 57
3.4.2 Freezing phase 58
3.4.3 Accuracy relative to chance performance 59
3.4.4 Concentration of samples 59
3.4.5 Conclusions 59
Chapter 4 General Discussion 60
4.1 Real World Significance 60
4.1.1 Biomass Limits 60
4.1.2 Sample preservation 61
4.1.3 Comparison to current aquatic species detection methods 62
4.1.4 Advantages of laboratory over field 63
4.2 Limitations 64
4.2.1 Use of volunteer pet dogs 64
4.2.2 Motivation of the dogs 64
4.2.3 Standard dilution in biomass limit experiment 65
4.3 Future research 65
4.4 Conclusions 66
References 67

Appendices 75

Appendix A: Standard Operating Procedure – Training Dogs for Scent Detection Work Using Automated Apparatus 75

Appendix B: Standard Operating Procedure – Acid Washing for Fish Projects 81

Appendix C: Session-by-session Data for Biomass Limit Experiment 85

Appendix D: Session-by-session Data for Preservation Experiment 88

List of Figures

Figure 1.1: Brown bullhead catfish (*Ameiurus nebulosus*). Photo credit: Noel Burkhead........3

Figure 2.1: The dogs that participated in the experiment, from left to right; Cobie, Mika, and Tommy........15

Figure 2.2: Scent detection self-operating apparatus front view........16

Figure 2.3: Scent detection self-operating apparatus birds eye view without lid........17

Figure 2.4: Feeders, a) Treat & Train b) Pet Tutor Pro c) Purpose-built wooden feeder........17

Figure 2.5 : Mika indicating on a positive sample........21

Figure 2.6: Tommy activating the omni-directional switch........22

Figure 2.7: Left, experiment tanks for holding catfish and goldfish, and a control tank. Water could drain from the top of the tanks if continuous flow-through was provided or completely drain from beneath each tank for cleaning purposes. Right, catfish in an experiment tank under constant flow-through conditions at the University of Waikato's Aquatic Research Centre........23

Figure 2.8: Daily mean percent correct by Cobie for standard catfish samples (Positive), standard goldfish samples (Goldfish), control samples (Control), and each catfish probe dilution (Probe), with the probe dilution value indicated within each phase........30

Figure 2.9: Daily mean percent correct by Mika for standard catfish samples (Positive), standard goldfish samples (Goldfish), control samples (Control), and each catfish probe dilution (Probe), with the probe dilution value indicated within each phase........31

Figure 2.10: Daily mean percent correct by Tommy for standard catfish samples (Positive), standard goldfish samples (Goldfish), control samples (Control), and each catfish probe dilution (Probe), with the probe dilution value indicated within each phase........32

Figure 2.11: Session data for Cobie, Mika, and Tommy when their percent correct for standard catfish samples (Positive), standard goldfish samples (Goldfish), and control samples (Control) were above 80%, with the probe sample (Probe) dilution value indicated within each phase........35

Figure 2.12: Cobie, Mika, and Tommy's hit rate (± 95% confidence interval) at each dilution and the hit rate that would be expected by chance performance. *Sessions once Mika and Tommy switched to Possyum........37

Figure 2.13: Cobie, Mika, and Tommy's correct rejection rate (± 95% confidence interval) at each dilution and the correct rejection rate that would be expected by chance performance. *Sessions once Mika and Tommy switched to Possyum........47

Figure 3.1: Daily mean percent correct for Cobie, Mika, and Tommy during each phase of the preservation experiment........51

Figure 3.2: Mean percent correct for each sample type (Positive, Goldfish, Control) for Cobie, Mika, and Tommy during each phase of the preservation experiment ± standard error. 52

Figure 3.3: Cobie, Mika, and Tommy's hit rate (± 95% confidence interval) at each phase of the preservation experiment and the hit rate that would be expected by chance performance. 55

Figure 3.4: Cobie, Mika, and Tommy's correct rejection rate (± 95% confidence interval) at each phase of the preservation experiment and the correct rejection rate that would be expected by chance performance. 56

List of Tables

Table 2.1: Summary information of dogs participating in the catfish scent-detection research. 14

Table 2.2 : Catfish probe sample water concentrations and equivalent biomass concentrations 25

Table 2.3: Possible outcomes from responding to a signal....26

Table 2.4: Number of sessions completed until Cobie, Mika, and Tommy first met criteria at each dilution. The experiment ended before Cobie and Tommy met criteria at their final dilution 28

Table 2.5: Mean hit rate of probe samples, and mean correct rejection rates of goldfish and control samples at each dilution for Cobie, Mika, and Tommy....33

Table 2.6: d' values for the biomass limit experiment 36

Table 3.1 Mean hit rate of positive samples, and mean correct rejection rates of control and goldfish samples during each phase of the preservation experiment.....53

Table 3.2: d' values for the preservation experiment 54

Chapter 1
Introduction

1.1 Freshwater Ecosystems

Freshwater ecosystems cover 0.8% of the Earth's surface, but contain significant global biodiversity, partially illustrated by the fact that approximately one third of all vertebrate species live in freshwater systems (Dudgeon et al., 2006). They also provide humans with drinkable water, food, recreation, and economic benefits (Dudgeon et al., 2006; Yick et al., 2021). Introduced organisms can have adverse effects on freshwater ecosystems and the native species that inhabit them by way of competition, predation, hybridisation, disease, and habitat alteration (Collier & Grainger, 2015; Collier et al., 2017; Dudgeon et al., 2006; Vitule et al., 2009). For example, the New Zealand mud snail (*Potamopyrgus antipodarum*) is an invasive species in Europe, Japan, Australia, and North America (Levri et al., 2007). In some rivers and streams, population densities can reach ~300,000 per m^2, outcompeting native grazers and reducing colonisation rates by native invertebrates (Kerans et al., 2005; Levri et al., 2007). Common carp (*Cyprinus carpio*) are one of the most extensively introduced freshwater fish species in the world and have been widely linked to the deterioration of water quality through bioturbation and macrophyte reduction (Adámek & Maršálek, 2013; Córdova-Tapia et al., 2015; Rowe, 2007; Yick et al., 2021). The global cost of aquatic invasive species is estimated to have totalled US$345 billion and has increased exponentially over time (Cuthbert et al., 2021). Despite their importance, freshwater ecosystems are increasingly impacted by species introduced both intentionally for recreational and economic benefits, as well as accidentally through negligence.

1.1.1 New Zealand's freshwater ecosystems

New Zealand's freshwater ecosystems are particularly vulnerable to non-native fish establishment because of their clow freshwater fish diversity compared to continental aquatic ecosystems (McDowall, 2010; Townsend, 1996). With just over 50 native freshwater fish species, New Zealand has a number of underfilled ecological niches, many of which have since been taken advantage of by non-native species (Dunn et al., 2018; Neilson et al., 2004; Townsend, 1996). Disproportionately, there are 21 introduced freshwater fish species present

in New Zealand (Dunn et al., 2018), making up almost one third of all freshwater fish species. Common carp, rudd (*Scardinius erythrophthalmus*), goldfish (*Carassius auratus*), perch (*Perca fluviatilis*), and the brown bullhead catfish (*Ameiurus nebulosus)* are a few examples of introduced fish species that have been linked to the decline in quality of New Zealand's freshwater ecosystems (Collier & Grainger, 2015; Rowe & Wilding, 2012). The introduction of benthic feeding non-native fish such as common carp, brown bullhead catfish, and tench (*Tinca tinca*) has exacerbated the decline of many of New Zealand's shallow lakes as their feeding behaviours can re-suspend sediment, increasing turbidity. High turbidity reduces light availability to submerged macrophytes which reduces their ability to photosynthesise and leads to the collapse of submerged macrophyte beds, resulting in increased sediment resuspension (Adámek & Maršálek, 2013; Collier & Grainger, 2015; Rowe, 2007). Ultimately, this results in reduction of water quality, loss of habitat and disruption of food-webs leading to local loss of native biodiversity.

1.1.2 Brown bullhead catfish

The brown bullhead catfish, hereafter referred to as catfish, was introduced to New Zealand in 1877 (Champion et al., 2020). They are a freshwater species native to the southern United States and can be found in many habitats including rivers, streams, wetlands, ponds, and lakes (Champion et al., 2020; Collier & Grainger, 2015). They typically reach 300 mm in length and have a brown to dark-olive colouring, with eight distinctive whisker-like barbels around their mouths (Barnes, 1996) (Figure 1.1). They are carnivorous and often considered to be opportunistic in their feeding behaviour, including gastropods, cladocerans, and caddisflies in their diets (Barnes & Hicks, 2003; Collier & Grainger, 2015). Catfish are tolerant of temperature extremes, low oxygen levels, and even extended periods out of water provided they remain moist. Depending on acclimation, catfish can tolerate temperature ranges from 0°C to 38°C and dissolved oxygen levels as low as 0.2 ppm in winter (Collier & Grainger, 2015). In New Zealand, they usually spawn between September and December, with the males providing parental care for their broods, an uncommon behaviour among freshwater fish species (Blumer, 1985; Collier & Grainger, 2015). These characteristics make catfish effective at competing with New Zealand's native fauna, including the native shortfin eel (*Anguilla australis*) with whom they share a trophic overlap. As they share many of the same habitat and prey, catfish are likely a significant competitor with native shortfin eels' diet (Collier et al.,

2018). They have also been closely involved in the decline of native kōura (*Paranephrops* spp.) by both competing with them for resources and through predation by adult catfish (Barnes & Hicks, 2003; Collier & Grainger, 2015; Francis, 2019). As well as competing with native fauna, catfishes' benthic feeding habits stir up bottom sediments that can alter invertebrate communities, ecosystem processes, and nutrient availability (Champion et al., 2020; Collier & Grainger, 2015). There are few studies available that estimate the biomass of catfish in New Zealand lakes, however, it appears that for most shallow lakes in the lower Waikato region, catfish biomass is typically 10-20 kg/ha. However, catfish populations have been reported to exceed 66 kg/ha in lakes when there are few competing common carp (Hicks et al., 2017; Tempero et al., 2019).

Figure 1.1

Brown bullhead catfish (Ameiurus nebulosus). Photo credit: Noel Burkhead

1.1.3 Current aquatic species detection methods

Early detection of invasive aquatic species is critical to preventing their spread (Pyšek et al., 2020; Trebitz et al., 2017). This can be difficult due to the interconnectedness of aquatic ecosystems, but there are reports of successful containment and eradications. For example, an emergent population of common carp in Tasmania, Australia were successfully eradicated from

Lake Crescent through a long-tern containment and eradication programme that began in the 1970s (Yick et al., 2021). This was primarily due to the early detection of establishing populations. Upon detecting the invasive species, the lake was cut off from other water sources to prevent any spread of the fish and various methods were then used to successfully eradicate the fish (Yick et al., 2021).

There are numerous survey methods used to detect the presence of fish in freshwater bodies including visual surveys, electrofishing, netting and more recently, environmental DNA (eDNA). Visual surveys are generally low cost, but are less effective in bodies of water that have low clarity or are very deep and they also require some experience in the identification of fish (Bernard et al., 2013; Grane-Feliu et al., 2019). Electrofishing permits species selectivity, but it is limited to shallow littoral areas and there is the potential for fish injuries from the electrical field if the proper procedures are not followed correctly (Cardás et al., 2020; Yick et al., 2021). With netting, it is possible to survey large areas comparatively cheaply, but this method generally results in unwanted bycatch, injury, and even death of non-target species (Neilson et al., 2004; Yick et al., 2021). Electrofishing and netting surveys also produce physical specimens, allowing for collection of supporting information such as sex, age, and the condition of the fish (Trebitz et al., 2017). However, such survey methods often require specialist equipment or need specially trained personnel to carry out the work, which can be constrained by budgetary considerations, thus limiting the number of water bodies that can be surveyed (Neilson et al., 2004; Trebitz et al., 2017). Visual surveys, electrofishing, and netting are usually sufficient to estimate relative population sizes of fish that are conspicuous and/or abundant (Jerde et al., 2011). However, they are relatively ineffective when species are cryptic and/or scarce, such as when populations are establishing or near extirpation. False negatives resulting from these surveys may result in no management action and the subsequent establishment of an unwanted species.

Environmental DNA (eDNA) is a method of detecting the presence of organisms in the environment from the cells that they shed (Bohmann et al., 2014; Clusa & García-Vázquez, 2018; Trebitz et al., 2017). Species that are cryptic nature or in low abundances can often be detected by eDNA as the organism itself does not have to be caught or seen. It is also relatively labour and cost effective by comparison to traditional netting and electrofishing surveys

(Bohmann et al., 2014; Jerde et al., 2011). A study on two species of introduced Asian carp (*Hypophthalmichthys* spp.) in Chicago, USA demonstrated that eDNA required less effort to detect the presence of the target species than electrofishing (Jerde et al., 2011). A positive eDNA sample from one of the study sites, which took 0.174 person days to collect and process, prompted an electrofishing mission that took 93 person days to catch a single carp (Jerde et al., 2011). Another study from Japan showed that eDNA had greater sensitivity for the detection of the endangered Japanese eel (*Anguilla japonica*) than electrofishing (Itakura et al., 2019). At 61 of the study sites, electrofishing was able to collect at least one specimen, while eDNA from the target species was detected at 56 of those sites. An additional 35 study sites produced a positive eDNA result where no eel was obtained through electrofishing (Itakura et al., 2019). Another benefit to eDNA sampling is that the collection of water samples in the field requires little training or equipment, and can occur comparatively quickly (Itakura et al., 2019; Trebitz et al., 2017). However, drawbacks of eDNA include the inability to provide any information about individual fish such as their size, age, or sex, difficulty quantifying relative population abundance, and the cost of developing species-specific amplification primers (Trebitz et al., 2017). Furthermore, processing of eDNA requires specialist equipment, facilities and technical expertise, especially in the development phase (Bohmann et al., 2014). The most significant issue with eDNA sampling is the potential for false positives (Bohmann et al., 2014; Clusa & García-Vázquez, 2018). With conventional methods such as electrofishing and netting, there is no uncertainty if the species is present because you would have physically seen or caught some individuals. However, with eDNA there is the possibility that the DNA of your target species has resulted from cross contamination or cross amplification of non-target species DNA due to poor specificity (Bohmann et al., 2014; Trebitz et al., 2017). A false positive is undesirable because it may prompt management action in the form of unnecessary control or eradication measure, wasting resources. While eDNA can be more sensitive at low biomass compared to traditional methods, detection limits can be variable due to environmental factors such as temperature and the presence of suspended sediment or other organic substances that bind and degrade DNA (Bohmann et al., 2014).

The application of these detection methods is fundamental to aquatic conservation; they work adequately with species that are conspicuous and abundant, and advances in new technologies, such as eDNA, have promise. One of the fundamental limitations of these methods is that they are currently incapable of surveying large numbers of aquatic systems on a regular basis at low

cost. The use of scent detection dogs to rapidly assess water samples for invasive fish under laboratory conditions may provide a solution to this challenge.

1.2 Scent Detection Dogs

Domestic dogs (*Canis familiaris*) have been employed by humans for many centuries participating in a wide range of vocations, the majority of which utilise their extraordinary olfactory systems (Beebe et al., 2016; Bennett et al., 2020; Dahlgren et al., 2012). They have been trained to detect all kinds of scents including those belonging to missing people, explosives, cancer, and illnesses in humans and other animals (Beebe et al., 2016; Lit, 2009). Scent detection dogs have also been increasingly employed in conservation and biosecurity work over the last few decades (Becker et al., 2017; Beebe et al., 2016; Bennett et al., 2020). Conservation management plans and actions are considerably influenced by species monitoring which dogs can be trained to carry out (Becker et al., 2017; Grimm-Seyfarth et al., 2021; Smith et al., 2003). Dogs have been trained to detect a range of target scents to help monitor both native and introduced species (Becker et al., 2017; Duggan et al., 2011; Grimm-Seyfarth et al., 2021). For example, in Western Zambia, scent detection dogs were trained to detect cheetah (*Acinonyx jubatus*) scat to assist in population surveys (Becker et al., 2017). Cheetah numbers are declining in the wild but large carnivore populations are often difficult to monitor due to their wide-ranging habits, low densities, and elusive behaviour (Becker et al., 2017). The scent detection dogs' ability to survey large areas for cheetah scat in a comparatively short amount of time proved to be effective for estimating their population sizes and distributions (Becker et al., 2017). This type of information helps researchers to make informed decisions about where to concentrate conservation efforts. In the United States, a California based study demonstrated that the detection rate of San Joaquin kit fox (*Vulpes macrotis mutica*) scat by the poorest performing scent detection dogs was as good as experienced human observers, and the better performing dogs were substantially more successful (Smith et al., 2003). Not only did the dogs find considerably more scat samples than the human observers, but they found sufficient samples that they could be used to collect information on the kit fox populations (Smith et al., 2003). The successful utilisation of scent detection dogs in terrestrial conservation work is well established internationally, and has also been demonstrated in New Zealand conservation programmes (Browne et al., 2015; Gsell et al., 2010; Powlesland et al., 1995).

1.2.1 Conservation dogs in New Zealand

Globally, the first use of dogs in conservation was in New Zealand over 100 years ago when they were trained to detect kiwi (*Apteryx* spp.) and kākāpō (*Strigops habroptilus*) by Richard Henry (Beebe et al., 2016; Browne et al., 2015) in order to relocate them to predator free islands (Hill & Hill, 1987). In more recent times, use of scent detection dogs in conservation has been accompanied with technological improvements such as GPS devices and jackets with LED lights that help to locate the dogs in dense bush (Becker et al., 2017; Beebe et al., 2016; Cablk et al., 2008; Griffiths et al., 2015). In New Zealand, one of the main roles of scent detection dogs is to locate introduced mammalian predators (Cowan, 1992; Gsell et al., 2010), particularly those that have breached predator free islands (Bellingham et al., 2010). For example, a single Norway rat (*Rattus norvegicus*) was detected in a tracking tunnel on Motuihe Island in 2008. This prompted an immediate deployment of a scent detection dog team that tracked it down within 24 hours (Bellingham et al., 2010). Scent detection dogs are also regularly employed to locate native birds (Powlesland et al., 1995; Robertson & Fraser, 2009; Robertson et al., 2016), but can also be called upon to detect other animals such as tuatara (*Sphenodon punctatus*) and geckos (Browne et al., 2015). They are an invaluable resource that often locate individuals that would not be found otherwise and can provide an unbiased sample of populations (Powlesland et al., 1995; Robertson et al., 2016; Robertson & Fraser, 2009).

1.2.2 Scent detection dogs and water

The majority of the work carried out by scent detection dogs generally occurs in terrestrial settings but they have also been trained to work on water or with water samples. Dogs have been trained to locate human remains on land in search and research missions for many decades and this skill has since been transferred to the aquatic medium (Osterkamp, 2011; Tirmenstein & Freedline, 2018). With the help of a skilful handler and expert boat operator, specially trained dogs can determine the approximate location of a corpse beneath the water surface from the molecules volatised into the air above the water (Osterkamp, 2011). Dogs can also detect contaminants in wastewater, which gives the potential to survey many sites very quickly and direct further chemical analyses (Reynolds & Reynolds, 2018; Van De Werfhorst et al., 2014). For example, Van De Werfhorst et al. (2014) tasked dogs with detecting human waste in storm drains and between the two dogs, there were no false negatives. There were a few false positives but there could be various explanations for this including the fact that the dogs may be capable

of detecting the target scent at concentrations lower than the instruments used for confirmation (Van De Werfhorst et al., 2014).

In addition to working in the field, scent detection dogs have been trained to assess water samples in laboratory settings. Shelby et al. (2004) investigated dogs' ability to detect off-flavour compounds in catfish pond water. Catfish (undefined species) crops for human consumption can often be ruined by rapid, unpredictable blooms of cyanobacteria that result in the uptake of off-flavour compounds in the fish's flesh, making them unsuitable for harvest (Shelby et al., 2004). The off-flavour compounds produced by these blooms can be detected before they accumulate in the fish, but the chemical analyses required for achieving this are expensive and require highly specialised personnel. Instead, analysis of the fish is undertaken by human taste-testers which is not a suitable solution as, by this stage, the fish are already spoiled (Shelby et al., 2004). Shelby et al.'s (2004) study proved that scent detection dogs could successfully detect the off-flavour compounds in pond water samples at relevant concentrations. This approach meant that samples could be assessed by the dogs faster than chemical analyses (Shelby et al., 2004).

1.2.3 Dogs in aquatic conservation and biosecurity

Although the use of scent detection dogs in conservation work on land has been extensive, there are few reported instances of their use in aquatic conservation. One example which demonstrated their ability as the successful use of dogs to locate whale scat in ocean waters (Rolland et al., 2006; Wasser et al., 2017). In an initial study of the North Atlantic right whale (*Eubalaena glacialis)* by Rolland et al. (2006), the authors state that scat collection by human observers gave them a large amount of raw data, but these samples were only found opportunistically and there were insufficient specimens to complete statistical analyses. This prompted an investigation to evaluate whether scent detection dogs could assist in locating whale scat (Rolland et al., 2006). They found that the dogs were four times more efficient than the human observers and their work produced enough samples for the investigators' statistical analyses (Rolland et al., 2006). This success led to scent detection dogs being used in a further study of the North Atlantic right whale (Rolland et al., 2017) and a study on orca (*Orcinus orca*) (Wasser et al., 2017).

Scent detection dogs have also been employed for the detection of invasive mussels under both field and laboratory settings (DeShon et al., 2016; Sawchuk, 2018). Introduced zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis*) mussels have become problematic and difficult to eradicate in numerous lakes in North America largely due to their lack of natural predators (DeShon et al., 2016). To prevent the spread of these organisms, scent detection dogs have been used to search watercrafts in North America. The dogs work in tandem with their handler to enhance efficiency and ensure the highest possible chance of detecting the potential invader. They are faster at searching watercrafts and successfully detected more mussels when in trial runs than their human handlers (Sawchuk, 2018). Quagga mussels are tiny in the juvenile phase and their larvae are too small for humans to see with the naked eye. This, coupled with the fact that adult specimens are rare, led to an investigation into whether scent detection dogs could detect quagga mussel veligers in water samples (DeShon et al., 2016). While the design of the study could have been improved by immediate reinforcement of positive alerts and an improved scent line up that allowed more definite assessment of every sample, the researchers did find that the dogs could correctly identify samples with small numbers of veligers present (DeShon et al., 2016).

The use of scent detection dogs for maintaining biosecurity of aquatic systems remains limited and they could be an untapped resource. An example of this potential is the use of scent detection dogs to detect invasive aquatic species, such as common carp and catfish, in laboratory water samples. These studies found that dogs could detect and discriminate the scent of their target species in water samples (Collins, unpublished data; Little, 2020; Quaife, 2018; Seal, 2019). For example, a study by Little, (2020) confirmed that scent detection dogs could detect catfish in water samples at 9,300 kg/ha, and another ongoing study confirmed the detection of common carp in water samples under laboratory conditions at biomass concentrations of 9.2 kg/ha and in real-world water samples at biologically relevant concentrations of 310 kg/ha (Collins, unpublished data).

1.2.4 Preservation of odours samples

There is little published research investigating the effects of sample preservation on dogs' abilities to detect and discriminate samples. A number of studies do preserve their samples, but this is often through necessity and not as a part of the investigation. For example, in cancer

research, samples often have to be frozen as they are not easily sourced (Gordon et al., 2008; Pickel et al., 2004; Willis et al., 2004). In other cases, field samples have to be preserved to conserve the sample before they can be assessed by the scent dogs (Browne et al., 2015; Fukuzawa & Sasahara, 2019; Rutter et al., 2021). Despite the requirement for preservation in numerous studies, research into the effects of preservation on dogs' scent detection abilities is minimal. A recent study by Matthew et al. (2021) included preservation effects as part of their research into scent detection dogs' ability to detect giant bullfrog (*Pyxicephalus adspersus*). They tested four preservation methods and found that the dog could successfully detect the target scent after 6-months of preservation and the best method was from an aqueous dilution prepared from skin swabs that were then stored at 4°C. The effects of preservation were also recently studied by Needs et al. (2021) in regard to dogs' ability to detect and generalise the scent of tall daisy (*Brachyscome diversifolia*) in Australia. Two groups of dogs were separately trained on either dried or frozen plant samples and were then tested on both dried and frozen as well as fresh samples. Their results showed that the dogs were able to detect all three sample types regardless of which they were trained on (Needs et al., 2021). With the increasing use of dogs in conservation and biosecurity, the preservation of samples is likely to become a more crucial part of the process.

1.2.5 Limitations of scent detection dogs

As with any detection method, employing dogs can have a range of advantages and disadvantages. Some benefits to employing dogs in monitoring and research have already been discussed, including their ability to cover large areas very quickly, their ability to locate cryptic species, and how they are often quicker than other analyses that require specialised personnel and instruments (Becker et al., 2017; Dahlgren et al., 2012; Duggan et al., 2011; Powlesland et al., 1995; Van De Werfhorst et al., 2014). However, the training of qualified scent detection dogs is expensive and time consuming (Duggan et al., 2011; Gsell et al., 2010; Long et al., 2007; Robertson & Fraser, 2009). A significant issue concerning working with dogs is the potential for them to cue off of their handlers (Browne et al., 2015; Edwards, 2019; Lit et al., 2011). Many studies attempt to eliminate cueing by ensuring that the handler does not know the position of the target samples (DeShon et al., 2016; Rutter et al., 2021; Van De Werfhorst et al., 2014). However, as demonstrated by Lit et al.'s (2011) study, human handlers can unintentionally cue their dog even when the study is double-blind. In Lit et al.'s (2011) study, handlers were informed that there would be up to three target scent locations present per

condition and that some of these would be visually marked. This was untrue and no target scents were in fact present but decoy scent locations were marked in some of the conditions. They found that the dogs were more likely to show an alert response to the marked decoy locations than other unmarked locations, suggesting that the handlers' belief that a target scent was present influenced the dogs response (Lit et al., 2011). Also with double-blind studies, the rewards are often delayed because the handler does not immediately know the status of the sample which can cause confusion for the dog (DeShon et al., 2016). Another element to consider is that although scent detection dogs are generally highly trained, they can become fatigued, lose motivation, or even injure a target species in the field (Dahlgren et al., 2012; Hill & Hill, 1987; Powlesland et al., 1995).

1.2.6 Resolutions

Whether the tool used to detect the presence of a target species is mechanical or canine, there will always be limitations, but there are measures that we can put in place to minimise them. Cost can be a significant issue, but this is not exclusive to dogs and their employment often works out be more cost effective when considering time taken and labour executed (Dahlgren et al., 2012; Gsell et al., 2010; Van De Werfhorst et al., 2014). Other studies have also shown that volunteer pet dogs are capable of achieving more than satisfactory results (Browne et al., 2015; Rutter et al., 2021). The potential for cueing has been eliminated in studies that have used an apparatus that is operated entirely by the dog participants working in isolation from their handler (Edwards, 2019; Little, 2020). Although dogs can become fatigued or bored, many working dogs are capable of physical activity for hours with only minimal rest periods (Becker et al., 2017; Gsell et al., 2010; Wasser et al., 2004). While this is not something that can be entirely controlled for, ensuring that working dogs get enough rest, and have appropriate rewards often keeps them motivated.

1.3 Project Aim and Thesis Structure

This research examined if scent detection dogs could be used to detect invasive catfish in water samples for the purpose of biosecurity monitoring programmes. Biologically relevant biomass concentrations are concentrations at which the target species is known to occur naturally; if scent detection dogs were ever to be employed in catfish monitoring, they would need to be capable of detecting at these levels and lower. Additionally, in practice, water samples

collected in the field are unlikely to be assessed by dogs immediately following collection. Hence, it is important that the dogs can maintain their ability to detect catfish in samples that have been preserved, and that the dogs can discriminate this catfish scent from the odour of other fish. Two experiments were conducted to investigate these subject matters.

The first experiment aimed to investigate whether scent detection dogs were able to detect the presence of catfish in water samples at biologically relevant biomass concentrations while discriminating against a distractor fish scent. This is presented in Chapter 2. The second part of this research investigated whether the preservation of water samples had an effect on the dogs' detection abilities. This is reported in Chapter 3.

Chapter 2
Biomass Limits for Scent-detection of Catfish

2.1 Introduction

Current survey methods for invasive fish species are often time-consuming and expensive which can restrict their use and make it difficult to manage invasive fish populations (Tempero et al., 2019; Trebitz et al., 2017; Yick et al., 2021). Improved monitoring frequency and sensitivity could help prevent catfish and other invasive fish species from establishing populations in freshwater systems. In order for any detection method to be employed in real-world situations it needs to be able to detect their target species below naturally occurring population limits. Therefore, an investigation into scent detection dogs' ability to detect catfish in water samples needs to confirm whether they can do so at biomass concentrations equivalent to establishing catfish populations.

Few robust estimates of catfish populations have been carried out in New Zealand as the required mark-recapture surveys are resource intensive. Catfish biomass has been reported at approximately 36 kg/ha in Lake Ohinewai from a mark recapture study and above 66 kg/ha from boat electrofishing surveys in other lakes, both occurring when few competing common carp were present (Hicks et al., 2017; Tempero et al., 2019). Biomass estimates from boat electrofishing likely underestimate abundance but ranges of 10-20 kg/ha are often reported for Waikato lakes. To be effectively employed for monitoring, dogs would need to be able to detect catfish at these biomass concentrations at a minimum. Dogs have already demonstrated their ability to detect catfish in water samples (Little, 2020).

The aim of Experiment 1 was to determine whether scent detection dogs can detect catfish odour at biologically relevant biomass concentrations (38.8 kg/ha or lower) while discriminating between other odours. It was hypothesised that the dogs would successfully reach biologically relevant biomass concentrations.

2.2 Methods

2.2.1 Animal ethics statement

Experiments using domestic dogs and invasive fish (catfish and goldfish) were carried out with the approval of the University of Waikato Animal Ethics Committee (protocol #1055). The dogs were housed in their own individual crates when they were not working or being walked. For every 2-hours that the dogs were in the laboratory, they were walked for 10-minutes, and they were taken for an additional 30-minute walk at midday if they were in the laboratory for the entire day. The dogs were walked by either a member of the Scent Detection Research Group or by trained volunteers. Participation was entirely voluntary, and owners were able to choose to remove their dog from the study at any time. Fish were housed at the University of Waikato Aquatic Research facility under University of Waikato SOP # 6 and SOP # 7.

2.2.2 Recruitment and selection of dogs

The three dogs, Cobie, Mika, and Tommy (Table 2.1, Figure 2.1) that participated in the experiments had already been recruited and trained to use the apparatus by a previous student. Other dogs were recruited for the project, with the aim of them joining the experiment, through word of mouth, and advertisements at the University of Waikato, local vets, doggy day care centres and online. Owners were required to fill out initial forms to assess whether the dog would be suitable. If they were believed to have potential, they were brought into the dog lab for an initial interview, and provided this went well, they could start basic training.

Table 2.1

Summary information of dogs participating in the catfish scent-detection research

Dog	Breed	Sex	Age (approximately at start)
Cobie	Labrador retriever x	Female	9 years
Mika	Border collie x springer spaniel	Female	2 years
Tommy	Border collie x springer spaniel	Male	2 years

Figure 2.1

The dogs that participated in the experiment, from left to right; Cobie, Mika, and Tommy

2.2.3 Fish collection and general maintenance

Fish were collected via boat electrofishing, with catfish sourced from Lake Rotoroa (Hamilton Lake), and goldfish from Lake Karapiro, south of Hamilton. All fish were held in flow-through tanks at the University of Waikato's Aquatic Research Centre. Catfish were housed in 230 L fibreglass tanks and goldfish were held with common carp in a 5000 L fibreglass tank. The tanks were supplied with flow-through dechlorinated Hamilton municipal water and oxygenation was maintained by continuous supply of compressed air fed through bubblers. Catfish tanks were supplemented with artificial seawater to a concentration of 5 ppt in order to reduce osmotic stress. Fish were fed twice a week during the warmer months and once a week during the rest of the year with commercial trout pellets. Any fish showing signs of ill health were removed from the experiment and either treated or euthanised if necessary.

2.2.4 Experiment apparatus

The scent-detection apparatus (Figure 2.2, Figure 2.3) was built at the University of Waikato and designed to be independently operated by the dog (Edwards, 2019). It consists of 17 stainless-steel numbered segments into which the odour samples are placed; the segments then

rotate on a carousel for individual assessment by the dog (Figure 2.3). A stainless-steel lid is placed on top of the segments and the dogs access the segment through a flap which sits behind a piece of clear Perspex with a circular hole in the centre (Figure 2.2). Some dogs had trouble finding the port because of the clear Perspex so a black framing border made of tape was added. On either side of the apparatus there is an omni-directional switch (lever), either of which the dogs can activate to reject a sample and cause the carousel to rotate to the next sample.

Figure 2.2

Scent detection self-operating apparatus front view

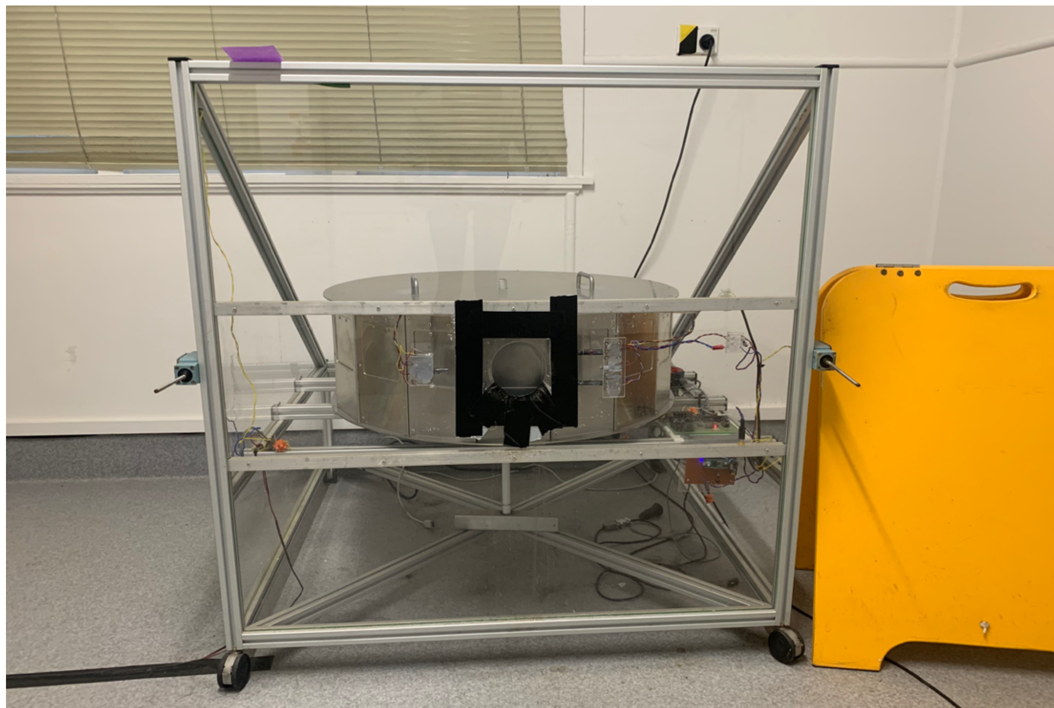
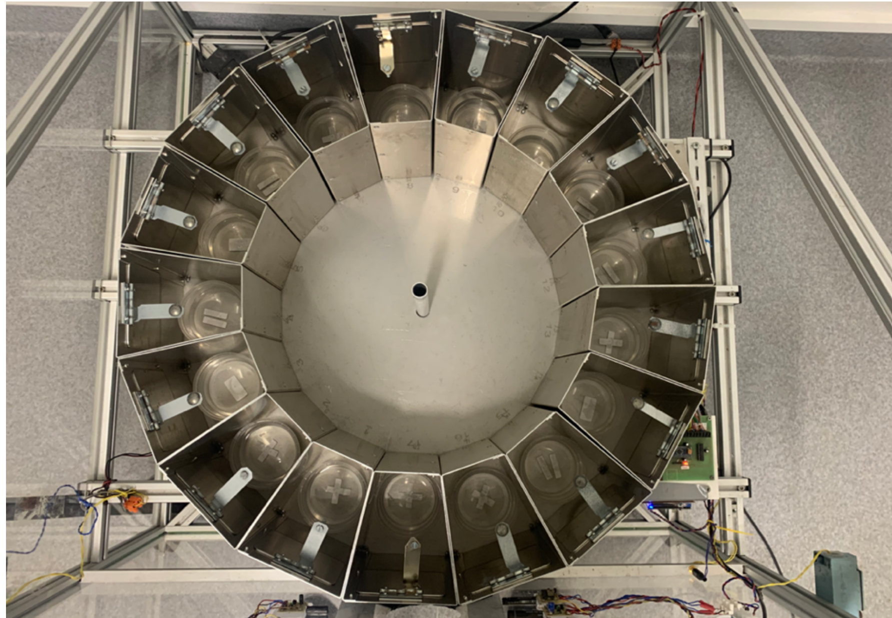


Figure 2.3

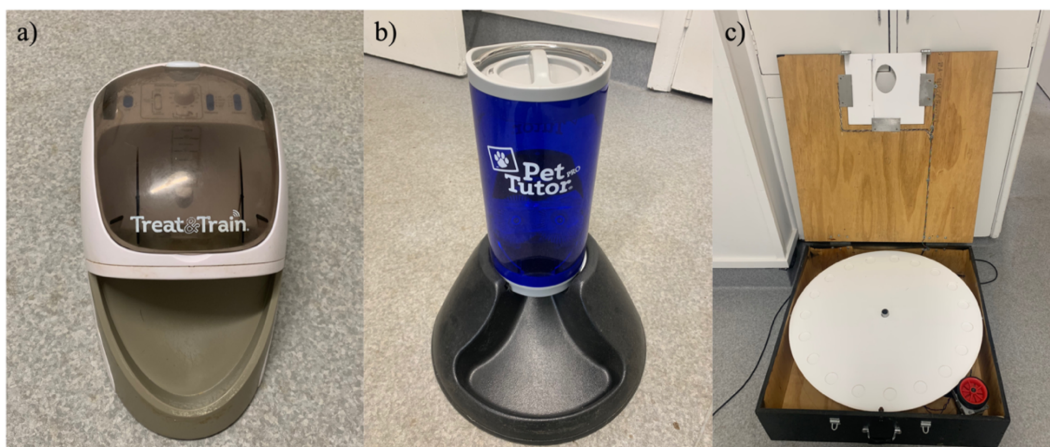
Scent detection self-operating apparatus birds eye view without lid



Three different automated feeders were trialled with the scent-detection apparatus over the duration of the experiments (Figure 2.4). The first was a Treat & Train feeder from PetSafe that dispensed dry kibble, this was only used for a few weeks before it was replaced by a Pet Tutor Pro feeder from Smart Animal Training Systems that was able to dispense wet food (Possyum dog roll). However, there were a number of problems with this feeder, prompting the introduction of a third purpose-built feeder (Figure 2.4c).

Figure 2.4

Feeders, a) Treat & Train b) Pet Tutor Pro c) Purpose-built wooden feeder



2.2.5 Dog training

Cobie, Mika, and Tommy had undergone training from a previous study and were already able to use the apparatus on their own to detect catfish at high concentrations, Cobie at 77,400 kg/ha, and Mika and Tommy at 9,300 kg/ha (Little, 2020). However, there were periods of time when they would not complete entire sessions, and thus some retraining was required.

Two dogs that were recruited with the intention of joining the project had to be released for various reasons. Chilli was excluded because he would not press the lever after assessing a control sample. Belle initially made good progress and reached the discrimination stage of training, but after a break for a month following minor surgery, she did not progress further through the training programme due to poor motivation.

2.2.6 Training procedure

Training the dogs to use the scent detection apparatus was accomplished through shaping, which is the differential reinforcement of successive approximations towards a target behaviour (Edwards, 2019). For each step in the shaping process, the dog had to achieve the current target behaviour three times in a row before progressing to the next target behaviour. Prompting was used occasionally but was always faded out before continuing to the next step. It was important to prompt with a consistent cue to ensure that there was no confusion in what was being asked of the dog. This was carried out for both Belle and Chilli prior to their withdrawal, and it is the same training that the three dogs in the experiment received.

During the initial training, the apparatus was switched off so that it did not make sounds or light up and a single, empty segment was placed at position one. The first step in the training procedure for shaping was to get the dog to associate the sound of the feeder with a food reward. After hearing the feeder activate and approaching it from anywhere in the work room, they could progress to the next stage which was shaping to put their nose into the apparatus port hole. The dog was reinforced for moving closer to the apparatus and gradually shaped to move towards the centre of the apparatus. Next, they received reinforcement for putting their nose into the port and then for touching their nose to the segment flap. Once the dog was reliably placing their nose into the segment, a positive sample containing 100 mL of undiluted catfish

water (15.5 g/L) was placed in the segment and the apparatus was switched on. The computer configuration file, which controlled the apparatus, was adjusted to have a minimum observation time of 500 ms and an indication time of 1000 ms. The observation time was the minimum amount of time that the dog had to hold their nose in the apparatus port for the computer to recognise that the dog had assessed the sample. The indication time was the amount of time the dog had to hold their nose in the apparatus port to indicate a sample as positive. The apparatus would make a beeping sound when the dog placed their nose into the port. The dog was manually reinforced for holding their nose in the port until they reached 1000 ms on their own, at which point the apparatus would trigger the feeder to dispense a reward. This was gradually increased until the dog was indicating for approximately 2500 ms. The next step was to shape the dog to press the omni-directional switch/lever. The behaviour for this action differed between dogs, some pressed the lever with their nose, others bit it with an open mouth. Once the dog activated the switch 10 times without any prompts, they could proceed with training. The final training step was for the dogs to combine the sample sniffing and lever pressing together to successfully discriminate between samples. A positive sample was placed at position one and a control sample was placed at position two on the apparatus. A full set of 17 samples was not required for training. The configuration file was set up so that the apparatus alternated between the positive and control samples to present the dog with a total of 17 samples. The dog was brought into the work room and the trainer waited for them to interact with the apparatus. If this didn't occur within 20 seconds the dog was prompted to smell the first sample. When they encountered their first control sample, the trainer waited for a few seconds before prompting them to the switch. Prompts were often needed for a short while during this stage of training until the dog could reliably press the switch with no prompts after smelling a control sample. Once a run of 17 samples was completed with no prompts, the order that the samples were presented to the dogs was randomised in the configuration file. This randomisation order was used for up to four carousel rotations before it was changed. The dogs worked with the samples until they reached a hit rate (percent of positive samples that the dog indicated on) and correct rejection rate (percent of negative samples that the dog did not indicate on) above 80%. While the dog was successfully working, the trainer slowly made their way out of the room until the dog was working on their own. The indication time was also gradually increased to approximately 4500 ms. This threshold was different for each of the dogs and the period in training when it was increased was not always the same. Some dogs naturally held their nose in the port for a long time which meant they had no issues with indication time increasing. Once the dogs were successfully discriminating positive and control

samples and working on their own, goldfish samples were introduced. A complete description of the shaping protocol is available in Appendix A.

2.2.7 Experiment design

This was a probe design experiment which involved a standard dilution at which the majority of the samples remained throughout the experiment with the addition of three "probe" samples which were included at increasingly lower dilutions. The benefits this design include were that the lower dilutions could be reached more quickly; if all of the samples were at very low dilutions, it would take considerably longer for the dogs to meet criteria. Also, the gradual lowering of all the samples would induce a 'learning' period whereby the dogs would become familiar with the scent before progressing to the next dilution step. Although this can still happen to some degree in a probe design experiment, the standard dilution remaining high helps to alleviate this as the probe samples were considerably lower.

For this experiment, the 17 available sample slots on the apparatus were assigned to three control samples, five goldfish, six standard catfish and three probe (i.e., diluted) catfish samples. The placement of these samples on the apparatus was determined through random number generation each day. Each session included two rotations of the apparatus for a total of 34 samples presented to the dogs per session. The criteria for success were that two out of three sessions had to have an accuracy (or percent correct) above 80% for each sample type. These criteria demonstrated that the dogs' accuracy with each sample type was above chance and occurred at least twice in three consecutive sessions. Dogs had to meet these criteria in order to progress to the next dilution. Dogs each completed a maximum of four sessions on any experiment day.

2.2.8 Experimental procedure

Experimental sessions for this project were generally conducted on Wednesdays and Thursdays each week. Dog owners were asked to not feed their dogs, or feed them a reduced amount, at least 2-hours prior to their arrival at the lab to ensure that they were motivated to work for food.

Each day, the position of the 17 samples on the apparatus was assigned through random number generation. This information was also entered into the configuration file for the scent programme to run on the apparatus. Each dog had their own indication time, which was adjusted if needed, but by the end of the project all three dogs were working at 4501 ms. If the dog indicated on a sample containing catfish, they were correct, the feeder beeped and reinforced this behaviour with food. The apparatus beeped and lit up green when the dog indicated on a positive sample (Figure 2.5). If they indicated on a negative sample, they were incorrect and had to press the lever for the apparatus to move onto the next sample.

Figure 2.5

Mika indicating on a positive sample

To reject a sample, the dog had to press the lever (Figure 2.6). If the dog sniffed the sample for a shorter amount of time than their indication time and pressed the lever, that was considered "not indicated". For negative samples, this was the required correct response, but for catfish samples this was incorrect. The dog was not reinforced for correctly rejecting negative samples or for any incorrect rejections or indications.

Figure 2.6

Tommy activating the omni-directional switch

The dog's response (indicated or not indicated) was recorded on a response sheet next to each sample number. Other parameters of interest were recorded including, start and end time of the sessions, and the temperature and humidity of the working room. At the end of each session an accuracy percentage was calculated for each sample type as well as for all positives (hit rate) and all negatives (correct rejection). Notes about the performance of the dog or any issues with the apparatus were also made.

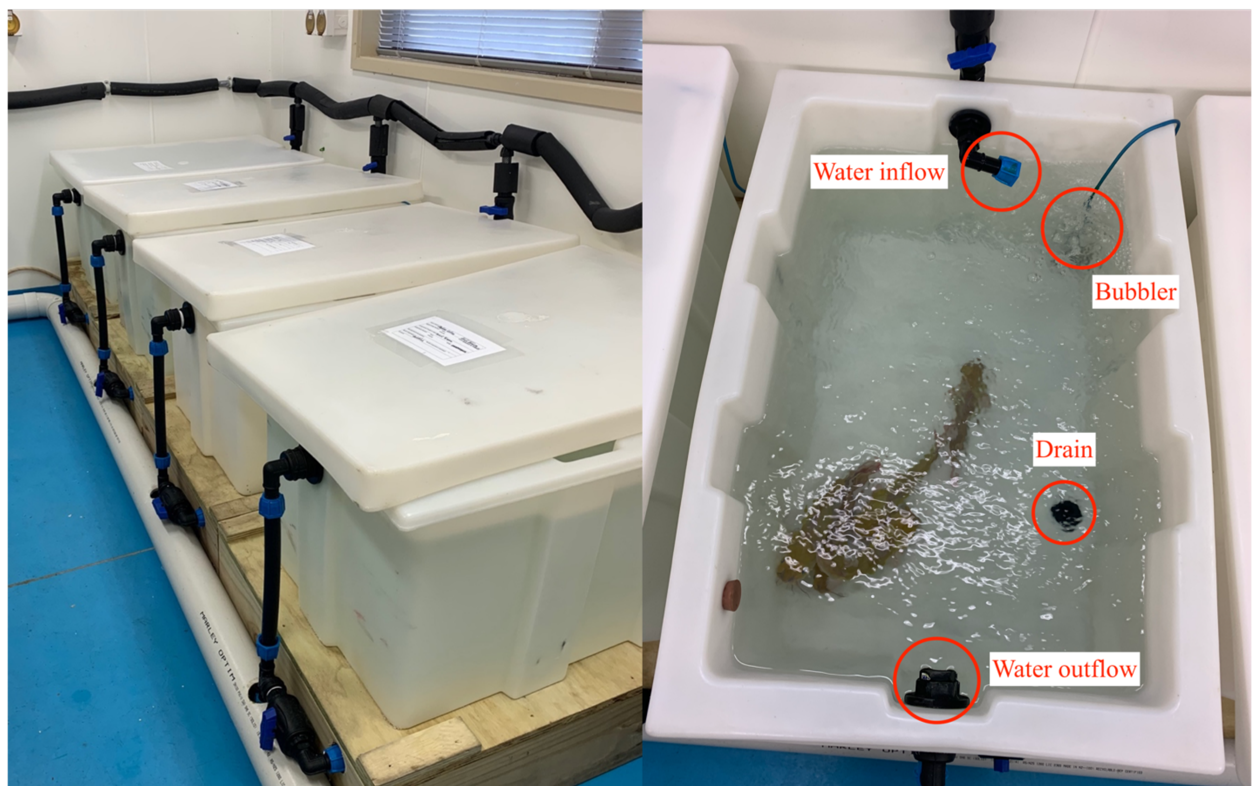
2.2.9 Sample collection and preparation

Catfish and goldfish were transferred from the holding tanks to the experimental tanks, which consisted of a control tank with no fish, a goldfish tank, and a catfish tank. The tanks were constructed of high-density polyethylene (HDPE) and covered with a corresponding HDPE lid to prevent cross contamination (Figure 2.7). Maximum tank volume was 200 L, and each tank was provided with flow-through dechlorinated Hamilton municipal water at 0.5 L/minute and oxygenation was maintained by continuous supply of compressed air fed through bubblers. Groups of 4-6 fish were held in the experiment tanks for 4-week periods, after which they were returned into their respective holding tanks; this was done to prevent the dogs from learning the odour of individual fish, and to reduce stress on the fish themselves. In addition, the experimental tanks were randomised every 3-months to reduce the chance of the dogs learning

to cue on specific tank odours. During the monthly switch-over, the experimental tanks were completely drained, scrubbed with tank-specific sponges, and sprayed with 10% v/v hydrogen peroxide to remove accumulated organic compounds. After spraying, the tanks were left for 15-minutes before being thoroughly rinsed with dechlorinated water, refilled, and put on flow-through for at least 24 hours before fish were re-introduced. The outsides of the tanks were cleaned with Janola wipes.

Figure 2.7

Left, experiment tanks for holding catfish and goldfish, and a control tank. Water could drain from the top of the tanks if continuous flow-through was provided or completely drain from beneath each tank for cleaning purposes. Right, catfish in an experiment tank under constant flow-through conditions at the University of Waikato's Aquatic Research Centre



To ensure a consistent fish biomass concentration of 15.5 g/L in the water samples, the volume of water in each experimental tank was adjusted according to the total weight of the fish in the tank, and the fish were held at that level without flow-through for 24-hours prior to samples being collected for presentation to the dogs. The volume of the control tank was not altered, this is later referred to as 'standing' levels. After the 24-hour holding period, the tank water

was collected in separate species-specific labelled 1 L glass Schott bottles (control, goldfish, and catfish) using a glass beaker. The bottles were kept in clean, plastic zip-lock bags and handled with gloves, which were changed between each bottle type. Following the water sampling, the tanks were scrubbed with their designated sponges, re-filled with water, and either drained back to standing levels for another 24-hours if the experiment was planned to run the next day, or returned to maintenance flow-through of 0.5 L/minute.

Prior to samples being prepared the stainless-steel bench in the dog laboratory was sterilised by pouring boiling water over it twice, the bench was then dried with paper towels and wiped with 70% isopropyl alcohol (IPA). Gloves were worn at all times when preparing and handling samples and were changed between the preparation of each sample type. To minimise potential cross-contamination, the left-hand side of the bench was designated for negative (control and goldfish) samples only and the right-hand side for positive (standard catfish and probe) samples. Label stickers were used to mark the sample containers for the various sample types. Each container, including the controls, had a sticker of the same size to ensure any odours from them were the same for each sample.

During experimental runs, dogs were presented with a consistent sample volume of 100 mL and sample fish biomass concentrations were adjusted as required. Goldfish and standard catfish samples remained at the standard concentration of 38,700 kg/ha throughout the experiment. Standard samples were prepared by the addition of 12.5 mL of fish tank water to 87.5 mL of control water to give the total sample volume of 100 mL. Initial catfish probe dilutions (numbers 1-4, Table 2.2) were prepared to a total volume of 100 mL, while dilution numbers 5-11 were prepared by the addition of the required volume of fish tank water to 100 mL, this was done as the concentration error was deemed to be smaller than the volumetric error for a sample volume of 100 mL. The remaining sampled water was segregated by source and stored at 4°C until required. If the dogs were working at different dilutions, a new set of samples was prepared after the first dog/s had run their sessions. The first set of samples was always prepared for the dog/s on the lowest dilution. Cobie did not complete the dilutions in a stepwise manner, she participated in dilutions 1,6, and 8-10 (Table 2.2). The decision was made for her to skip some dilutions due to time constraints resulting from motivational issues, but this did not affect her performance.

Table 2.2

Catfish probe sample water concentrations and equivalent biomass concentrations

Probe dilution number	Fish water (µL)	Control water (mL)	Probe equivalent biomass concentration (mg/L)	Equivalent environmental biomass (kg/ha)
1	1500	98.5	232.5	4650
2	800	99.2	124.0	2480
3	400	99.6	62.0	1240
4	200	99.8	31.0	620
5	100	100	15.5	310
6	50	100	7.7	155.0
7	25	100	3.9	77.4
8	12.5	100	1.9	38.8
9	6	100	0.9	18.6
10	3	100	0.5	9.2
11	1.5	100	0.2	4.6

2.2.10 Cleaning

The stainless-steel segments were cleaned in warm water containing a dissolved dishwashing tablet. The segments were then rinsed with tap water and immersed in a 50% isopropyl alcohol (IPA) bath before being left to air dry. The scent-detection apparatus was wiped with 70% IPA using paper towels including the base of the apparatus, the underside of the lid, the front face of the apparatus, and the omni-directional switches.

The 1 L glass bottles were washed in separate sample specific 10% v/v hydrochloric acid (HCl) baths. Bottles were left to sit for at least 12 hours before being rinsed three times with reverse osmosis (RO) water and placed in a drying oven at 45°C. The glass containers, glass measuring cylinders, glass beakers, and glass funnels were washed in concentrated nitric acid for at least 12 hours then rinsed three times with RO water before oven drying. Full descriptions of acid washing procedures are available in Appendix B.

2.2.11 Statistical analysis

The dogs could respond to a sample as either yes (indicated) or no (not-indicated) and this produced various outcomes depending on whether the signal (target odour) was present (Table

2.3). A daily mean percent correct was calculated for each sample type for each individual dog, which was displayed in a series of line graphs. Sensitivity or hit rate (HR) was calculated for the standard positive and probe samples using the equation HR = TP/TP + FN. Specificity or correct rejection rate (CRR) was calculated for the goldfish and control samples using the equation, CRR = TN/TN + FP.

Table 2.3

Possible outcomes from responding to a signal

	Yes Response	No Response
Signal Present	Hit True Positive (TP)	Miss False Negative (FN)
Signal Absent	False Alarm False Positive (FP)	Correct Rejection True Negative (TN)

The mean number of sessions taken to meet criteria at any dilution across all of the dogs was calculated along with the mean hit rate and correct rejection rate at each dilution. Standard error (SE) was calculated for each of these means. If a dog was performing at chance levels, they would be equally likely to indicate on positive and negative samples. As the dogs in this study did not indicate on the same number of total samples at each dilution, chance performance was calculated for individual dogs. Individual expected chance hit rate and chance correct rejection rate were calculated from the proportion of positive indications to the number of positive and negative samples. For example, if there were 20 (25%) positive samples and 60 (75%) negative samples and the dog indicated positive 24 times, these indications would be allocated proportionately to each sample type in the form of hits or false alarms. Hence, 25% (6) are allocated to the positive samples, designated as hits, and 75% (18) are allocated to the negative samples designated as false alarms. The resulting hit rate chance performance would be 30% (6/20) and the false alarm rate (percent of negative samples that the dog indicated to be positive) chance performance would also be 30% (18/60). The correct rejection rate is the inverse of the false alarm rate which would be 70% (42/60). Therefore, if a dog had a higher hit rate than 30% their performance would be above chance and the equivalent for correct rejection rate. The expected chance performance for each dog at each dilution was then compared to the 95%

confidence interval calculated for the hit rate and correct rejection rate. A dog was considered to have performed above chance levels if the value at the lower 95% confidence interval limit was greater than the calculated value of expected chance performance.

Individual d' values were calculated for each of the dilutions using the equation d' = z(H) - z(F), where H = hit rate and F = false alarm rate (Stanislaw & Todorov, 1999). For this experiment, separate d' values were calculated using the standard catfish sample hit rate and the probe hit rate to provide a better understanding of how the dogs were assessing the two positive samples. The false alarm rate was calculated using both control and goldfish samples for all calculations. Therefore, each dog had two d' values for each of their dilutions. A d' value of above 0 suggests that a participant can distinguish signals from noise and the highest calculable value is considered to be 4.65 which would indicate near perfect performance (Macmillan & Creelman, 2005; Stanislaw & Todorov, 1999). As d' cannot be calculated from values of 0 or 1, any case where a dog achieved a perfect hit rate (1.0) this value had to be transformed to an estimated value. The equation $(n - 0.5)/n$ was used, where n is the number of sessions (Macmillan & Creelman, 2005; Macmillan & Kaplan, 1985). This estimation can result in values much lower than 1 when very few sessions were conducted at that dilution, however, this process did not considerably affect the calculated d' values. All values that had to be estimated are marked in their respective table.

An additional visual analysis was conducted with the data from the sessions where the dogs achieved above 80% accuracy for each of their standard catfish, goldfish, and control samples. This was done to evaluate the dogs' accuracy with the probe samples when they were performing well with all other samples. These sessions were displayed in separate graphs for comparison.

Microsoft Excel was used to create all graphs and complete all statistical analyses.

2.3 Results

All participants detected catfish in probe samples at concentrations <20 kg/ha, while simultaneously discriminating between catfish, goldfish, and control samples at higher concentrations. All participants detected catfish at dilutions lower than the highest relevant biomass concentration (38.8 kg/ha), Cobie achieved criteria at 18.6 kg/ha, Tommy at 9.2 kg/ha, and Mika at 4.6 kg/ha which was the lowest dilution that was tested in this study.

The mean number of sessions required to meet criteria across all three participants was 8.4 (± 1.51 SE) (Table 2.4). Sessions where Mika and Tommy were working without Possyum were excluded as it was unlikely that they would have met criteria if the reinforcer was not changed. The minimum number of sessions that a dog could spend on a dilution was two as this would satisfy the two out of three sessions accuracy criteria without the need for the third session. This was achieved five times, twice by Mika and three times by Tommy. The longest time spent on any of the dilutions was 26 sessions by Tommy at 155 kg/ha. Cobie did not complete all of the dilutions and those that she skipped are marked in their various tables.

Table 2.4

Number of sessions completed until Cobie, Mika, and Tommy first met criteria at each dilution. The experiment ended before Cobie and Tommy met criteria at their final dilution

Dilution (kg/ha)	Cobie	Mika	Tommy
4650	3	30^{+}	38^{+}
4650*	-	7	3
2480	-	9	3
1240	-	11	4
620	-	4	2
310	-	7	2
155	22	13	26
77.4	-	6	6
38.8	4	2	2
18.6	21	2	4
9.2	16^{+}	16	24
4.6	-	8	10^{+}

*Sessions once Mika and Tommy switched to Possyum.

$^{+}$ The dog did not meet criteria at this dilution.

2.3.1 Accuracy

Mean accuracy across all sessions for each day are provided for Cobie (Figure 2.8), Mika (Figure 2.9), and Tommy (Figure 2.10); with the full session-by-session data presented in Appendix C. In general, the dogs had high hit rates (>80%) with both the positive catfish and probe samples (Figure 2.8, Figure 2.9, Figure 2.10), but they also regularly had low correct rejection rates with the goldfish, and particularly the control samples. On meeting criteria, the accuracy of control samples regularly declined when a new dilution was introduced and the dogs' accuracy with the negative samples varied considerably.

The hit rate for all the dogs' standard catfish samples was consistently at or near 100%. The mean hit rate for probe samples and mean correct rejection rates for control and goldfish samples at each dilution is displayed in Table 2.5. Mika and Tommy's probe hit rate was also consistently higher compared to Cobie. In contrast, Cobie often had higher correct rejection rates for her control samples than Mika and Tommy who had a wide range of control correct rejection values. At some dilutions they achieved a perfect 100% and at others, it was well below 50%. All three dogs had similar correct rejection rates for their goldfish samples.

For logistical reasons, dogs were not always immediately moved onto the next dilution after meeting criteria. Therefore, the change to a lower dilution is not always preceded by the dog achieving above 80% accuracy for each sample type. In these cases, the dog had met criteria in earlier sessions but had to continue at that dilution until another dog met criteria. The sessions where the dogs first met criteria at each dilution are marked on the graphs in Appendix C.

Figure 2.8

Daily mean percent correct by Cobie for standard catfish samples (Positive), standard goldfish samples (Goldfish), control samples (Control), and each catfish probe dilution (Probe), with the probe dilution value indicated within each phase

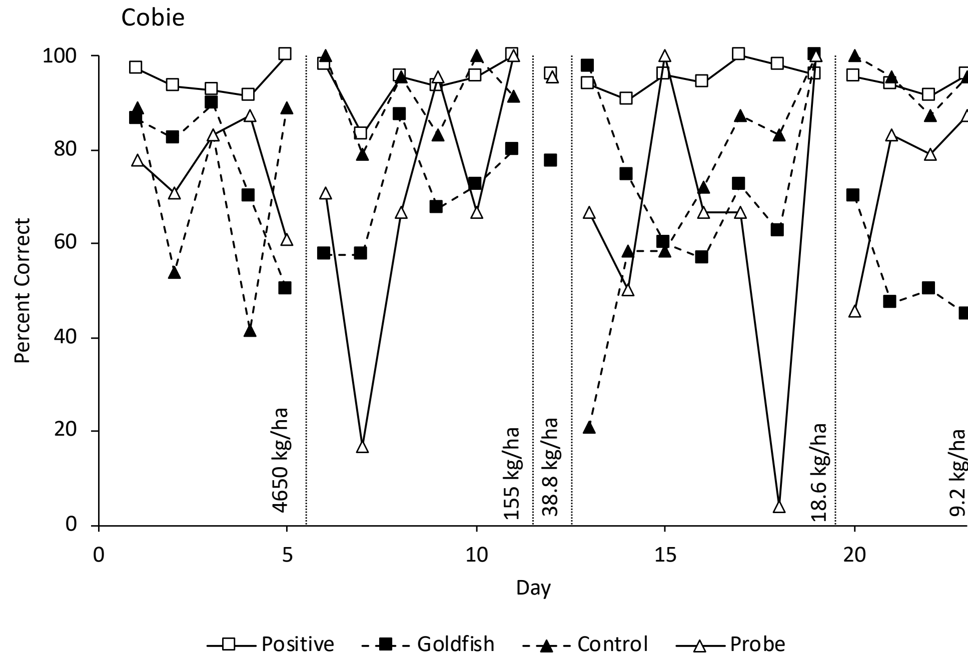


Figure 2.9

Daily mean percent correct by Mika for standard catfish samples (Positive), standard goldfish samples (Goldfish), control samples (Control), and each catfish probe dilution (Probe), with the probe dilution value indicated within each phase

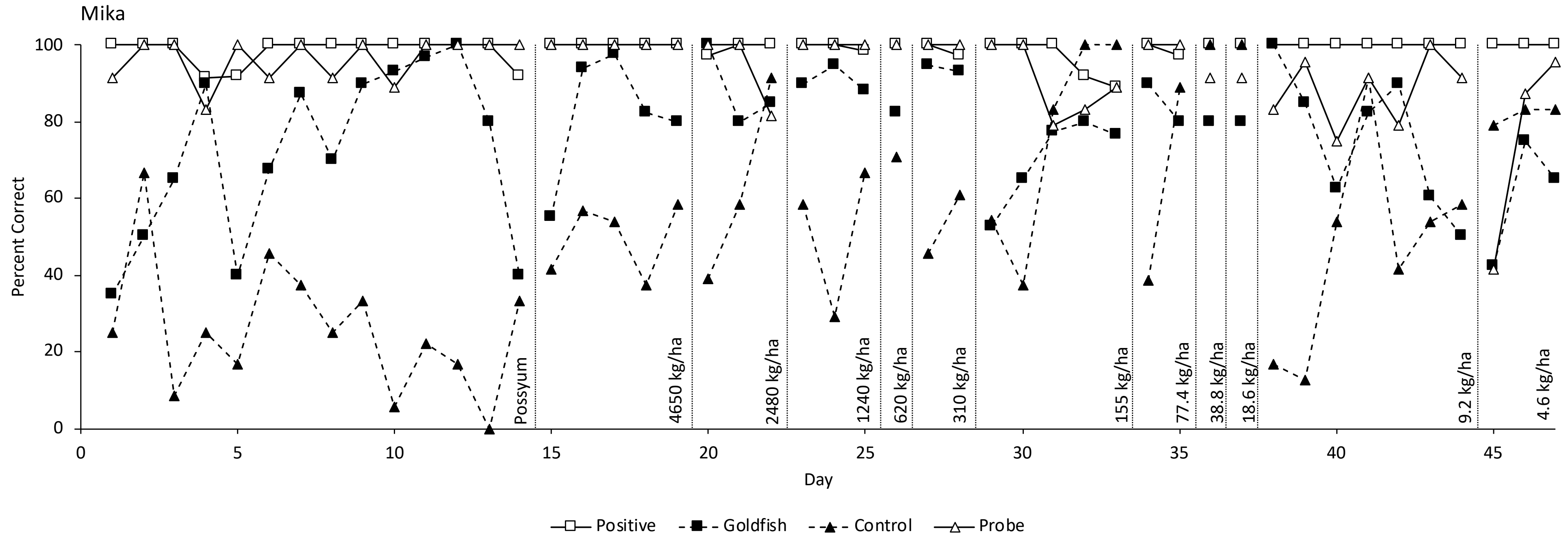


Figure 2.10

Daily mean percent correct by Tommy for standard catfish samples (Positive), standard goldfish samples (Goldfish), control samples (Control), and each catfish probe dilution (Probe), with the probe dilution value indicated within each phase

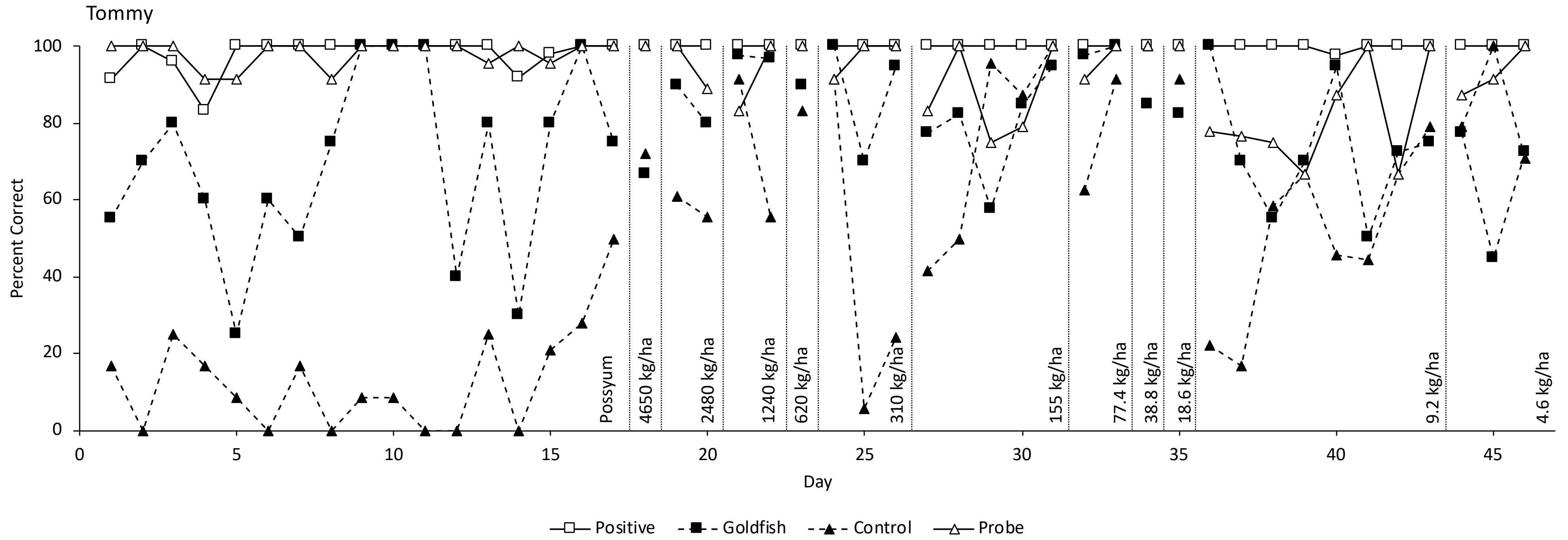


Table 2.5

Mean hit rate of probe samples, and mean correct rejection rates of goldfish and control samples at each dilution for Cobie, Mika, and Tommy

Dilution	Probe Hit Rates				Control Correct Rejection Rates				Goldfish Correct Rejection Rates			
(kg/ha)	Cobie	Mika	Tommy	Mean ± SE	Cobie	Mika	Tommy	Mean ± SE	Cobie	Mika	Tommy	Mean ± SE
4650	77%	97%	98%	91% ± 6.98	69%	37%	19%	42% ± 14.73	77%	78%	71%	75% ± 2.05
4650*	-	100%	100%	100% ± 0.00	-	50%	72%	61% ± 11.11	-	86%	67%	76% ± 9.61
2480	-	96%	94%	95% ± 0.89	-	59%	58%	59% ± 0.46	-	88%	85%	86% ± 1.39
1240	-	100%	90%	95% ± 4.76	-	52%	76%	64% ± 12.34	-	91%	97%	94% ± 3.12
620	-	100%	100%	100% ± 0.00	-	71%	83%	77% ± 6.25	-	83%	90%	86% ± 3.75
310	-	100%	98%	99% ± 0.93	-	52%	32%	42% ± 10.15	-	94%	88%	91% ± 3.25
155	67%	92%	87%	82% ± 7.70	91%	69%	71%	77% ± 7.11	70%	69%	75%	71% ± 2.00
77.4	-	100%	94%	97% ± 2.78	-	64%	72%	68% ± 4.17	-	85%	98%	92% ± 6.67
38.8	96%	92%	100%	96% ± 2.41	96%	100%	100%	98% ± 1.39	78%	80%	85%	81% ± 2.20
18.6	60%	92%	100%	84% ± 12.34	67%	100%	92%	86% ± 9.76	75%	80%	83%	79% ± 2.32
9.2	74%	88%	83%	82% ± 4.15	95%	47%	50%	64% ± 15.45	53%	76%	75%	68% ± 7.53
4.6	-	75%	93%	84% ± 7.48	-	82%	80%	81% ± 0.79	-	61%	69%	65% ± 3.33
All Dilutions	70%	93%	93%	85% ± 7.65	81%	53%	51%	62% ± 9.70	70%	78%	78%	75% ± 2.72

*Sessions once Mika and Tommy switched to Possyum.

2.3.2 Final sessions

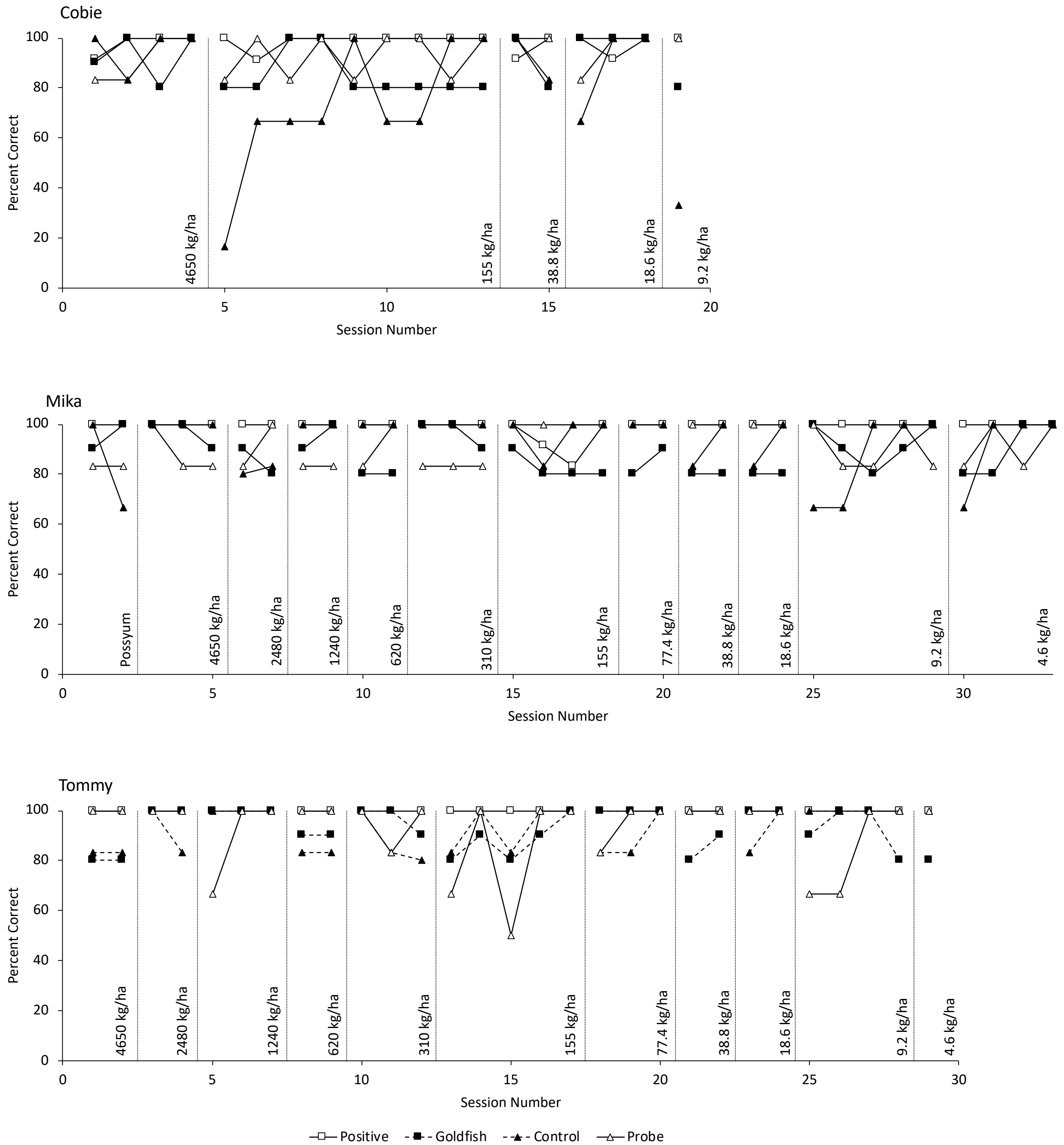
In four of Tommy's sessions at his lowest dilution of 4.6 kg/ha, for which he did not meet criteria, he achieved above 80% for all samples except the goldfish. In his last two sessions of the experiment, he was 100% accurate with each of his standard positive, control, and probe samples but only reached 70% for his goldfish samples. On Cobie's last day of the experiment, she was working at 9.2 kg/ha, for which she also did not meet criteria. Her accuracy for each of the standard positive, probe, and control samples were above 80% but she did not achieve 80% accuracy or above for the goldfish samples.

2.3.3 Probe accuracy in high accuracy sessions

To evaluate the dogs' probe accuracy during sessions in which they were performing well with all other sample types, only sessions when the dogs achieved above 80% accuracy with each of these other samples (i.e., standard catfish, goldfish, and control) were selected for this additional visual analysis (Figure 2.11). The dogs' accuracy with their probe samples was consistently high in this subset of "high accuracy" sessions compared to when all sessions are considered where there is a lot more variation.

Figure 2.11

Session data for Cobie, Mika, and Tommy when their percent correct for standard catfish samples (Positive), standard goldfish samples (Goldfish), and control samples (Control) were above 80%, with the probe sample (Probe) dilution value indicated within each phase



2.3.4 d' calculations

The d' values for the data calculated using only the standard catfish samples hit rate and the data including only the probe samples hit rate are displayed in Table 2.6. Approximately two thirds of the values are above 2.0. All of the other values are above 1.0 except for Cobie's probe only data at 9.2 kg/ha.

Table 2.6

d' values for the biomass limit experiment

Dilution (kg/ha)	Cobie		Mika		Tommy	
	Standard	Probes	Standard	Probes	Standard	Probes
4650	2.32	1.38	2.77	2.20	2.16	2.17
4650*	-	-	2.49+	2.49+	1.46+	1.46+
2480	-	-	3.10	2.49	2.06+	2.23
1240	-	-	3.14	2.40+	2.71+	2.52
620	-	-	1.93+	1.93+	2.88	1.82+
310	-	-	3.05	2.26+	2.04+	2.50
155	2.30	1.20	2.43	2.13	2.69+	1.75
77.4	-	-	2.94	2.12+	2.59+	2.76
38.8	2.74	2.76	1.82+	2.56	1.99+	1.99+
18.6	2.33	0.83	1.82+	2.56	2.23+	2.23+
9.2	2.07	1.13	2.49+	1.56	2.47+	1.36
4.6	-	-	2.22+	1.16	2.26+	2.09
All	2.28	1.16	2.92	1.96	2.90	1.94

Standard: d' calculated using the hit rate of only the standard catfish samples.

Probes: d' calculated using the hit rate of only the probe samples.

*Dilutions once Mika and Tommy switched to Possyum.

+Values when d' had to be estimated due to 1.0 hit rates.

2.3.5 Accuracy relative to chance

Figure's 2.12 and 2.13 display the dogs' accuracy with 95% confidence intervals at each dilution and displays the accuracy that would be expected by chance. The 95% confidence intervals for each of the dogs at all of the dilutions do not cross over the calculated expected chance performance lines.

Figure 2.12

*Cobie, Mika, and Tommy's hit rate (± 95% confidence interval) at each dilution and the hit rate that would be expected by chance performance. *Sessions once Mika and Tommy switched to Possyum*

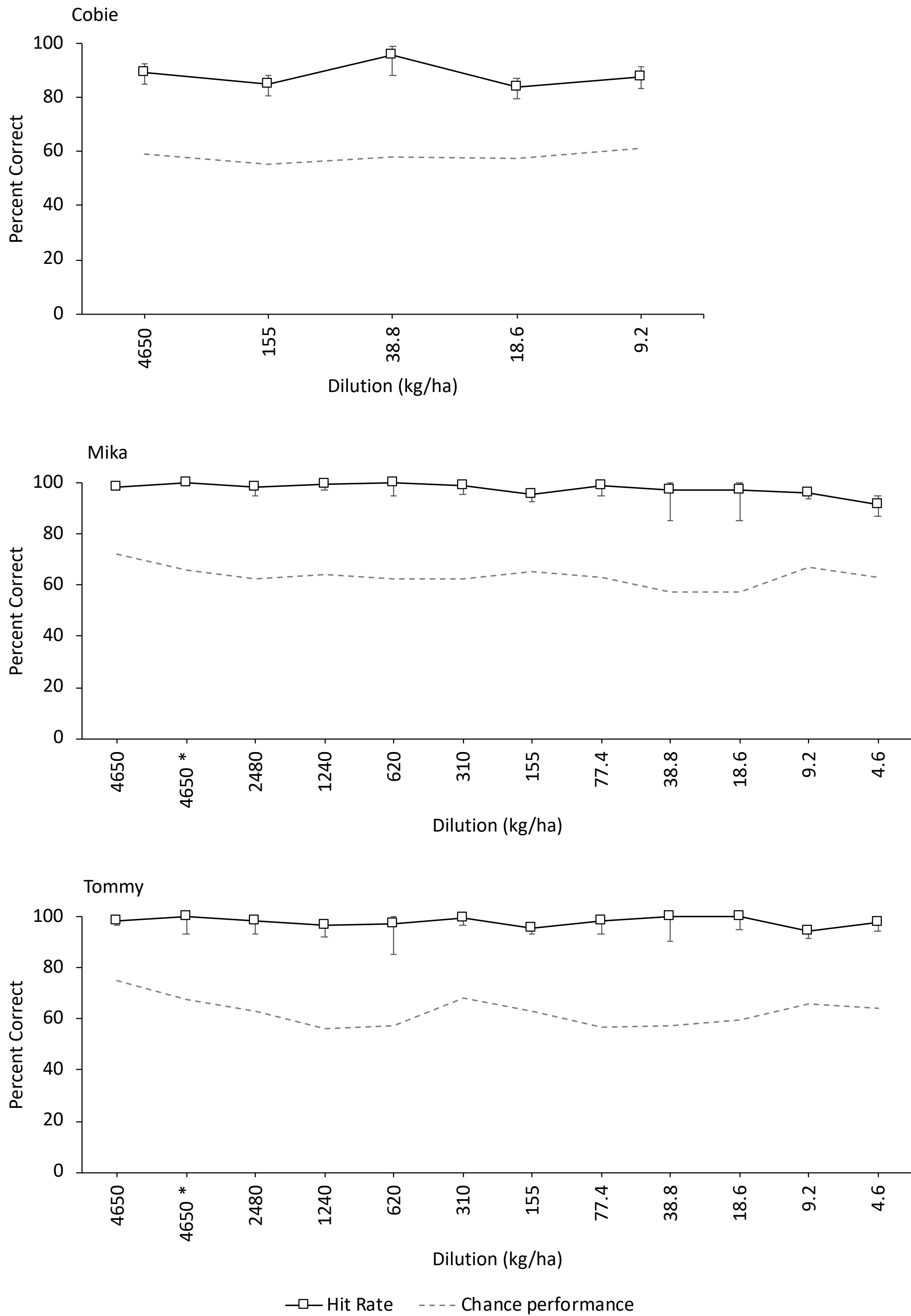
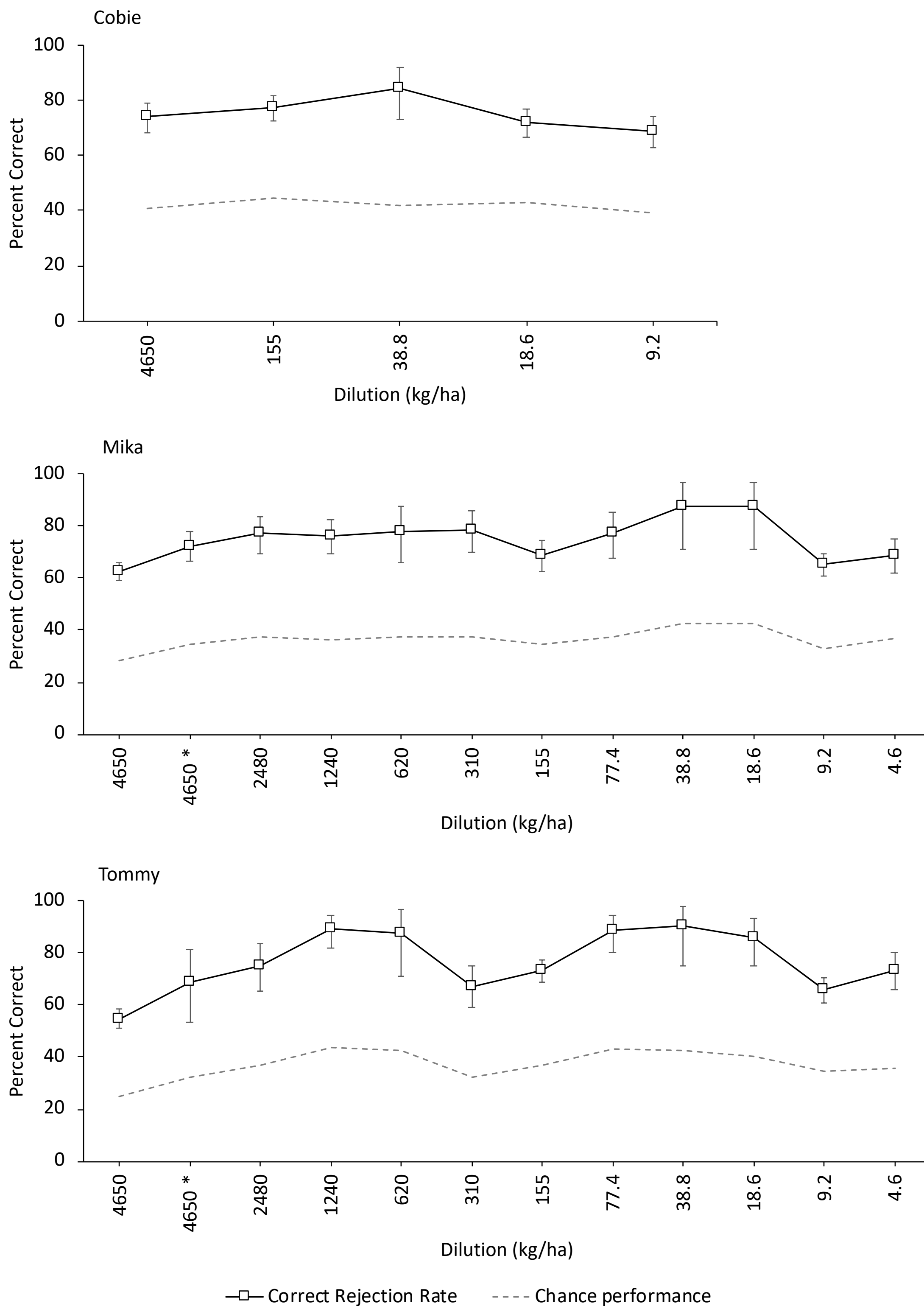


Figure 2.13

*Cobie, Mika, and Tommy's correct rejection rate (± 95% confidence interval) at each dilution and the correct rejection rate that would be expected by chance performance. *Sessions once Mika and Tommy switched to Possyum*



2.3.6 Introduction of Possyum

Mika and Tommy both had their reinforcer changed from kibble to Possyum which is marked on the graphs in Figure 2.9 and Figure 2.10 respectively and in Appendix C. Tommy appeared to have an already increasing trend in his control sample correct rejection rates before the Possyum was introduced. Tommy met criteria after only three sessions with the Possyum which is in contrast to the fact that he was on that same dilution working with kibble for 38 sessions (Table 2.4). Mika did not have an already increasing trend before the switch to Possyum (Figure 2.9) and similar to Tommy, she met criteria quickly after her reinforcer was changed (Table 2.4).

2.4 Discussion

2.4.1 Biological relevance

The aim of this study was to determine whether scent detection dogs are able to detect catfish odour in laboratory water samples at biomass concentrations ≤38.8 kg/ha while discriminating between non-target odours. Using a stepwise approach, the dogs successfully achieved the accuracy criteria of two out of three consecutive sessions with accuracy for each sample type above 80% at successively lower concentrations, ranging from 4650 kg/ha to 4.6 kg/ha. The dogs were able to successfully detect and discriminate the scent of catfish at the following concentrations: Cobie achieved 18.6 kg/ha, Tommy achieved 9.2 kg/ha, and Mika achieved the lowest concentration of 4.6 kg/ha. These concentrations are lower than most catfish population biomasses in lakes of the lower Waikato region (~10-20 kg/ha) (Tempero et al., 2019).

2.4.2 Session numbers

The number of sessions required for the dogs to succeed at each dilution ranged from two to 26 and at 155 kg/ha, which is in the middle of the dilutions that were tested, the dogs required considerably more sessions to meet criteria than for the dilutions immediately preceding and following. For example, Tommy needed two sessions at 310 kg/ha and six sessions at 77.4 kg/ha, but he needed 26 sessions to meet criteria at 155 kg/ha. This was also the dilution that Tommy needed the most sessions out of any of the dogs for any of the dilutions. This may be explained by the fact that this dilution immediately followed the Christmas break. The dogs had been away from the laboratory for four weeks and it is feasible that they needed time to adjust to their return. For the majority of the experiment, however, the dogs only required a small number of sessions to meet criteria at each dilution. A mean of only 8.4 sessions across all of the dogs, meant that the dogs often only required two experimental days on a dilution. There were also a number of days where Mika and Tommy would only need two sessions to complete a dilution which suggests that the dogs may have been able to generalise the target odour that they were familiar with as its scent profile changed slightly with the progressively lower dilutions (Cablk et al., 2008; Needs et al., 2021; Oldenburg et al., 2016).

2.4.3 Hit rates and correct rejection rates

All participants had little trouble detecting the standard catfish samples in the biomass limit experiment, but crucially they were able to discriminate the target scent from non-target scents. If scent detection dogs were to potentially be employed for aquatic system monitoring they would need to be capable of this (Bennett et al., 2020). Generally, the dogs had lower accuracy with the control and goldfish samples when first moving onto a new dilution, but this increased as they completed more sessions.

Mika and Tommy both consistently had high hit rates for their probe samples; both of their overall hit rates were above 90%. In comparison, Cobie regularly had a lower, but still acceptable, hit rate for these samples, with an overall hit rate of 70%. High hit rates for the probe samples means that they were successful at indicating on these samples most of the time and very rarely incorrectly rejected them at increasingly low concentrations. Cobie was on the biomass limit experiment for a much shorter amount of time than Mika and Tommy which could explain why she was unable to detect the probe samples as frequently as them. Often the more experienced a dog is with a scent, the better they are at detecting it (DeShon et al., 2016; Smith et al., 2003). It is possible that Cobie's comparative inexperience with the target scent in the probe samples led to fewer indications on these samples.

In contrast, while all three dogs' goldfish correct rejection rates were comparable, Cobie's correct rejection rates for her control samples were frequently much higher than those of Mika and Tommy. While Mika and Tommy achieved overall control correct rejection rates of just 53% and 51% respectively, Cobie achieved 81%. The probe and control samples would have been the most similar in scent once the probes reached low dilutions. It is possible that when the dogs could not detect a difference between these samples, they had different approaches to whether or not to indicate on them. While Cobie was rejecting these samples, Mika and Tommy were instead indicating on them. Further evidence that this is potentially the case is that when Mika and Tommy were working with the probes at their first dilution of 4650 kg/ha, which is a comparatively high dilution, they were indicating consistently on both probe and control samples until they were moved to Possyum. All of this suggests that while Mika and Tommy had higher hit rates with their probe samples, they were not necessarily any better at consistently detecting the presence of catfish in the probe samples than Cobie. To truly confirm

that the dogs could detect the probe samples, they had to be able to reject the control samples too. It often took Mika and Tommy a few sessions to meet the correct rejection rate criteria with the control samples, while it often took Cobie a few sessions to meet the hit rate criteria with the probe samples. A chemical analysis into the volatile organic compound (VOC) profiles of these samples could be beneficial to help quantify the difference between the control and probe samples (Collins, 2018; DeGreeff & Peranich, 2021; Forbes et al., 2014). This could help to provide an explanation as to why the dogs treated the two sample types quite differently. Despite the dogs' accuracy with these two sample types being quite different, they were all successful at discriminating between them, the goldfish, and standard samples at biologically relevant biomass concentrations.

2.4.4 Distinguishing signals from noise

A participant is considered able to distinguish signals from noise if the d' value is above 0 (Stanislaw & Todorov, 1999) and the higher the value the greater the ability; with the highest calculable value considered to be 4.65 (Macmillan & Creelman, 2005). The successful discrimination of signals from noise is important for the dogs in this study as they need to be able to identify the target scent (signal) in a sample while successfully discriminating these from the non-target scents (noise). This is especially critical for the potential future introduction of samples from lake water, which will likely have many more non-target scents than laboratory prepared samples (Oldenburg et al., 2016).

None of the calculated d' values were 0 which indicates that the dogs are able to distinguish signals from noise. However, Cobie's d' value for the 18.6 kg/ha dilution that was calculated using a hit rate of only her probe samples, was much smaller than the others and was less than 1 (0.83). This suggests that at that dilution Cobie was able to distinguish the probe samples from noise but not as well as at other dilutions.

The equation ($(n-0.5)/n$) that was used to transform the perfect 1.0 hit rates to an estimated value was not ideal because it meant that there was unequal treatment of the data points (Macmillan & Creelman, 2005; Macmillan & Kaplan, 1985). For example, due to some dogs requiring very few sessions to meet the accuracy criteria, the equation $(n-0.5)/n$, where n is

the number of sessions, estimated the hit rate of 1.0 to a value much lower. Values that were genuinely 0.99, often produced larger d' values than those of 1.0 because they did not have to be estimated. Regardless, all of the d' values that resulted from estimated hit rates were high enough to indicate the successful discrimination of signals from noise.

2.4.5 Accuracy relative to chance

The hit rates and correct rejection rates at the lower end of the 95% confidence interval range were all well above the calculated expected chance performance. This provides support for the assertion that the dogs were not indicating randomly whether the samples were positive or negative. This also validates the data where the dogs achieved the accuracy criteria at each of the dilutions because it demonstrates that the dogs were assessing the smell of the samples instead of randomly indicating or rejecting them. This is important in the context of Cobie's d' value at 18.6 kg/ha which was low enough to indicate that she was perhaps not consistently distinguishing signals from noise at that dilution because the results from this suggest that she was at least performing at levels above chance.

2.4.6 Probe accuracy in high accuracy sessions

The dogs performed well with their probe samples across all of the dilutions when they were achieving above 80% with the three other sample types. By examining probe sample performance in this subset of "high-accuracy" sessions, we gain a clearer understanding of the dogs' probe accuracy when parts of the experiment that could not be controlled for were potentially not having a significant impact. For example, the dogs' motivation on a particular day could affect their accuracy which is not entirely reflective of their catfish odour detecting abilities. The fact that Cobie's accuracy with the probe samples was much more similar to Mika and Tommy's accuracy with these samples when only this subset of data was considered suggests that this could be the case.

2.4.7 Change in reinforcer

Once their reinforcer was switched to Possyum, Mika's and Tommy's performance improved markedly, and they progressed comparatively quickly through the remainder of the dilutions. It appears that Tommy already had a somewhat increasing trend for his performance with control samples but when taking the previous 38 sessions into account, it seems possible that the switch to Possyum is what resulted in him meeting criteria. Mika had no obvious trend in her performance with control samples before the switch to Possyum and like Tommy, she had many sessions at 4650 kg/ha before being the switch. She worked for 30 sessions with kibble before the change was made and once she was working with Possyum, the maximum number of sessions she needed to meet criteria for the remained of the experiment was 16. Both dogs' motivation for Possyum appeared to be higher than it was for kibble which may have resulted in an increase in accuracy for their control samples. The design of this study means that it cannot be confirmed that Possyum was the exclusive reason for this because it is also possible that an extraneous variable coincided with the switch to the new reinforcer (Johnston et al., 2020). However, this happened with two dogs, and they were switched at different times, suggesting that Possyum could be the reason for their improved performance. Many studies use food as a reinforcer when training and working with dogs (Rutter et al., 2021; Shelby et al., 2004; Smith et al., 2016) and it has been established that dogs have variable preferences for their food (Tobie et al., 2015). Additionally, reviews that discuss traits to consider in working dogs often mention food drive (Beebe et al., 2016; Rooney & Bradshaw, 2004). Therefore, it is reasonable to expect that changing the food reinforcer received by these dogs could have had an impact but a detailed analysis of this was beyond the scope of this study, and no specific data collection or statistical analysis of this occurred. However, it may be of relevance to training dogs in the future and therefore could be worthy of further research.

2.4.8 Conclusions

This study has demonstrated that scent detection dogs are capable of detecting the presence of catfish in water samples at concentrations that are biologically relevant in a conservation context. The dogs were performing their assessment at levels well above chance and they were successful at distinguishing signals from noise. They are able to do all of this with high hit rates and correct rejection rates, and the results from this study indicate that the use of scent detection dogs may have potential for use in catfish and other aquatic species management programmes.

Chapter 3
Preservation Experiment

3.1 Introduction

Experiment 1 demonstrated that dogs are capable of detecting catfish at biologically relevant biomass concentrations. The highest dilution considered to be biologically relevant in this study was 38.8 kg/ha which was surpassed by all three dogs (Cobie 19.6 kg/ha, Tommy 9.2 kg/ha, Mika 4.6 kg/ha). This was critical for this research as it supports the continuation of the investigation into scent detection dogs in catfish detection. There are still many questions that need answering before dogs could be employed in conservation and biosecurity programmes, one of which concerns sample preservation.

As a conservation tool, water samples that are collected from the field would often not be assessed by the dogs immediately for a variety of reasons which could include the remote location of field sites or be a result of lengthy transportation to dog assessment facilities. Therefore, samples would likely require some method of preservation prior to assessment. Although dogs have been employed in scent detection work for centuries now (Beebe et al., 2016; Bennett et al., 2020; Dahlgren et al., 2012), the effects of sample preservation on dogs' abilities to detect odours have not been thoroughly explored. Despite this, studies involving scent detection dogs have been preserving samples but this has mostly been through necessity rather than through an interest into the effects of preservation (Fukuzawa & Sasahara, 2019; Rutter et al., 2021; Willis et al., 2004). Lake samples could potentially be presented for assessment by dogs in both fresh and preserved forms depending on sample storage duration. This suggests that an investigation into whether dogs could remain successful at detecting and discriminating catfish scent in preserved water samples is vital.

The aim of Experiment 2 was to determine whether the preservation of samples via refrigeration and freezing at a fish biomass of 310 kg/ha had an effect on the dogs' ability to detect and discriminate catfish odour in samples. It was hypothesised that the refrigeration of samples would negatively affect the dogs' detection abilities, but that freezing would not have an effect.

3.2 Methods

3.2.1 Animal ethics statement

The same protocols were carried out for this experiment as described in Chapter 2.

3.2.2 Recruitment and selection of dogs

Cobie, Mika, and Tommy were the only participants involved in this experiment.

3.2.3 Preservation experiment design

This experiment used a repeated-measures reversal design, which included an initial baseline, a refrigeration phase, a second baseline, and a freezing phase. A third baseline was planned but the August 2021 Covid-19 lockdown interrupted the experiment, preventing this baseline phase from being completed. The baseline phases serve as a control where there is no independent variable present, which is then compared to the following treatment phases where the independent variable is introduced (Johnston et al., 2020). For this experiment, the baseline phase had the dogs' assessing samples that had not been preserved and were prepared the same day that they were assessed, and the independent variable that was introduced was the preservation methods. The main benefit of a reversal design is that we can see if responding, or accuracy, returns to baseline levels after the treatment has been removed. If it does, we can be more confident that the treatment was the reason for the change in accuracy (Johnston et al., 2020).

To ensure that a change between phases occurred when the dogs were performing at a stable accuracy level, they had to meet stability criteria rather than criteria based solely on accuracy. The stability criteria required that the difference in average accuracy of two experiment days had to be within 10% of the average accuracy from the two previous experiment days for each sample type. For example, if a dog had an accuracy for catfish samples of 85% over two experiment days, the average accuracy for catfish for the next two experiment days could be as low as 75% but no higher than 95%.

These criteria were more difficult to meet than the criteria used in the previous experiment and required at least two weeks as only two experiment days occurred each week. Due to this, and time constraints, only one dog was required to meet criteria for the experiment to progress to the next phase. Cobie was chosen as the dog to base these criteria off as she had been performing the most consistently during the transitional stage when we were preparing for the preservation experiment. Despite this, Mika and Tommy often met criteria or were very close (within 2%).

For this preservation experiment, the dogs worked with fish samples that were diluted to 310 kg/ha. While on the biomass limit experiment described in Chapter 2, the dogs were working at a standard dilution of 38,700 kg/ha which is considerably higher. Therefore, a few weeks were allocated between the two experiments to gradually lower the dilution of these samples from 38,700 kg/ha to 310 kg/ha. This was not done in a stepwise manner as in the biomass limit experiment but with the use of only an additional two dilutions, 4650 kg/ha and 620 kg/ha. The dogs had no trouble with this and achieved criteria at 310 kg/ha in less than a month.

3.2.4 Experimental procedure

The dogs assessed the samples as they did in the biomass limit experiment. However, in this experiment, each session consisted of only one rotation of the apparatus for a total of 17 samples presented to the dogs per session. This meant that the number of sessions that the dogs completed on an experiment day was increased to eight.

3.2.5 Water sampling and sample preparation

The experimental tanks, preparation bench, and sample containers were prepared as previously described in the biomass limit experiment methods. Plastic sample containers were used in this experiment instead of glass as a way to minimise exposure to hazardous nitric acid. No effects on the dogs' performance were noted.

3.2.6 Collection and preparation of baseline samples

Control, catfish, and goldfish baseline samples (fish biomass concentration 310 kg/ha) were collected and prepared the same day that they were presented to the dogs using procedures previously described in Chapter 2.

3.2.7 Collection and preparation of refrigerated samples

The Wednesday the week before the samples were to be assessed, water was collected from the experimental tanks at the Aquatic Research Centre for both the following Wednesday and Thursday. Sample solutions were prepared in bulk quantities at biomass concentrations of 310 kg/ha for fish samples, and 100% control water for control samples. These were refrigerated at 4°C for 7 or 8 days before being assessed by the dogs.

On the experiment day, the sample solutions were removed from the fridge and inverted three times. 100 mL was then measured into each of the required number of sample containers using a clean measuring cylinder for each sample type.

3.2.8 Collection and preparation of frozen samples

Water was collected and prepared in the same manner as previously described for the refrigerated samples but were instead collected in 2 L plastic bottles to ensure enough headspace for the water to expand when freezing and these were placed in the freezer at -20°C for 6 or 7 days.

24 hours prior to an experiment day, the sample solutions were removed from the freezer and left to defrost at room temperature. On the experiment day, the sample solutions were inverted three times and 100 mL was measured into each of the sample containers using a clean measuring cylinder for each sample type.

3.2.9 Cleaning

Cleaning of equipment occurred as described in the biomass limit experiment. The 2 L plastic bottles used to store the frozen samples in this experiment were cleaned via the same methods as the 1 L glass bottles. The plastic sample containers were taken for recycling.

3.2.10 Statistical analysis

A daily mean percent correct was calculated for each sample type for each of the dogs and displayed in a series of line graphs. A mean percent correct was also calculated for each sample type for the entirety of each phase and displayed in a bar graph. Standard error was calculated for each mean. Chance performance levels and confidence intervals for the accuracy data were calculated and compared via the methods explained in the biomass limit experiment.

Hit rates, correct rejection rates, and d' were calculated as they were for the biomass limit experiment. No hit rate values had to be estimated as none of them were 1.0. A repeated measures ANOVA was calculated using the d' values to determine whether there was a significant difference between the preservation methods at the 95% confidence level.

Microsoft Excel was used to create all graphs and complete all statistical analyses.

3.3 Results

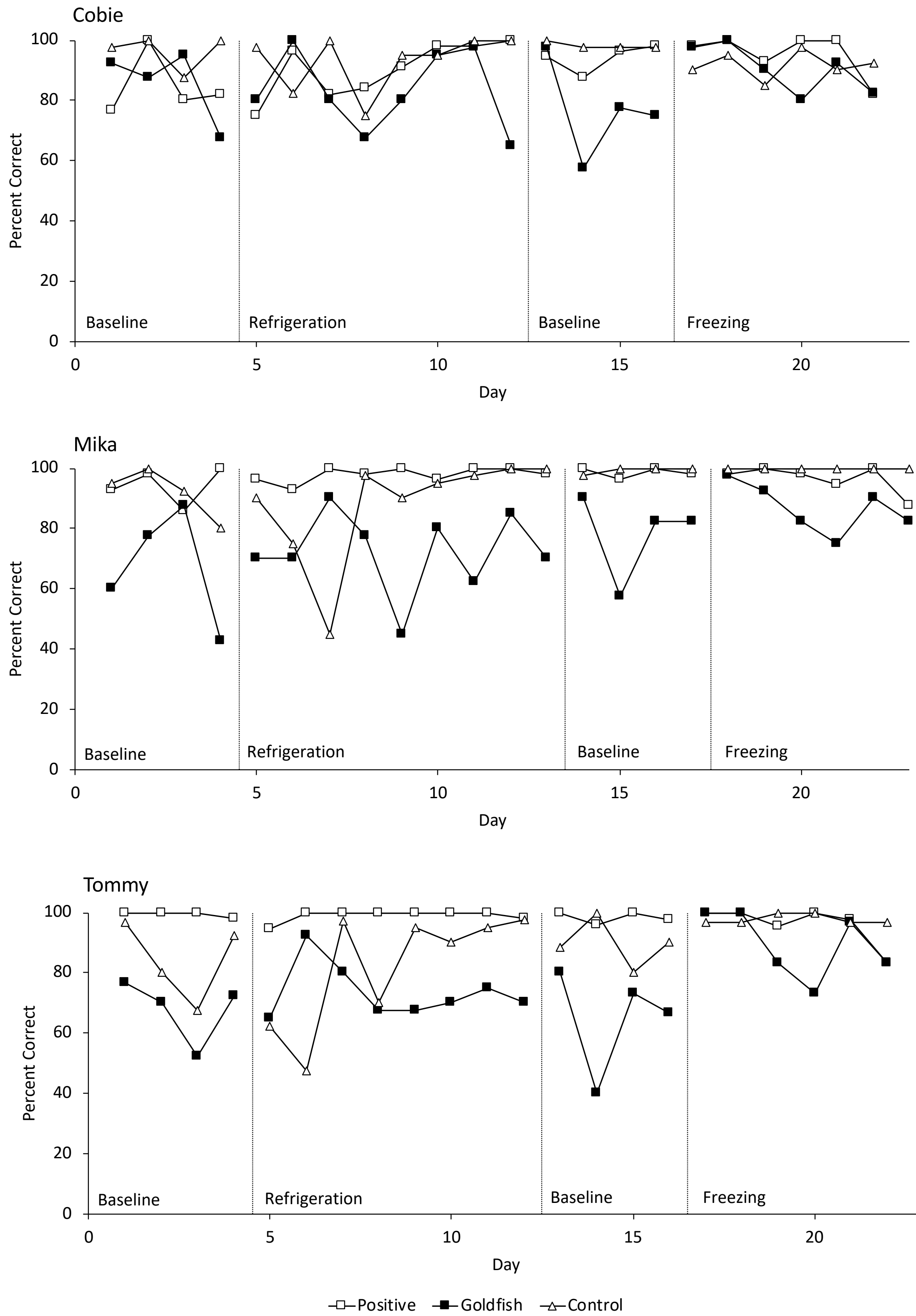
3.3.1 Daily means

All participants were able to maintain stable detection performance levels across the baseline and preservation phases. The preservation of samples did not have a significant impact of the dogs' ability to detect the target odour. Over 23 experimental days, each dog completed a minimum of 155 sessions. Unequal amounts of time were spent on each of the phases as stability criteria had to be achieved before progressing. The daily mean percent correct data displayed in Figure 3.1 shows that the dogs' accuracy remained high throughout the experiment for both the positive and control samples, while the accuracy for the goldfish samples was variable. Goldfish accuracy was visually more stable in the freezing phase as shown in Figure 3.1 and in the session-by-session data available in Appendix D. All of the dogs had high accuracy the first week that they worked on the frozen samples (Figure 3.1). For comparison, Cobie, Mika, and Tommy achieved a mean percent correct for goldfish samples in their first week on refrigerated samples of 90%, 80%, and 78.75% respectively. They subsequently achieved 98.75%, 95%, and 100% accuracy with their goldfish samples for their first week on frozen samples.

Cobie met the stability criteria before each of the phase changes, but she was the only dog to whom these criteria were applied. However, Mika also met the stability criteria at each phase apart from the initial baseline, while Tommy met criteria at the refrigeration phase and baseline 2.

Figure 3.1

Daily mean percent correct for Cobie, Mika, and Tommy during each phase of the preservation experiment



3.3.2 Summary accuracy results

The mean percent correct for each sample type during each preservation method is displayed in Figure 3.2. These varied slightly throughout the experiment but across all of the dogs there does not appear to be any obvious trend. The goldfish mean correct rejection rate was the highest for each of the dogs in the freezing phase (Figure 3.2, Table 3.1).

Figure 3.2

Mean percent correct for each sample type (Positive, Goldfish, Control) for Cobie, Mika, and Tommy during each phase of the preservation experiment ± standard error

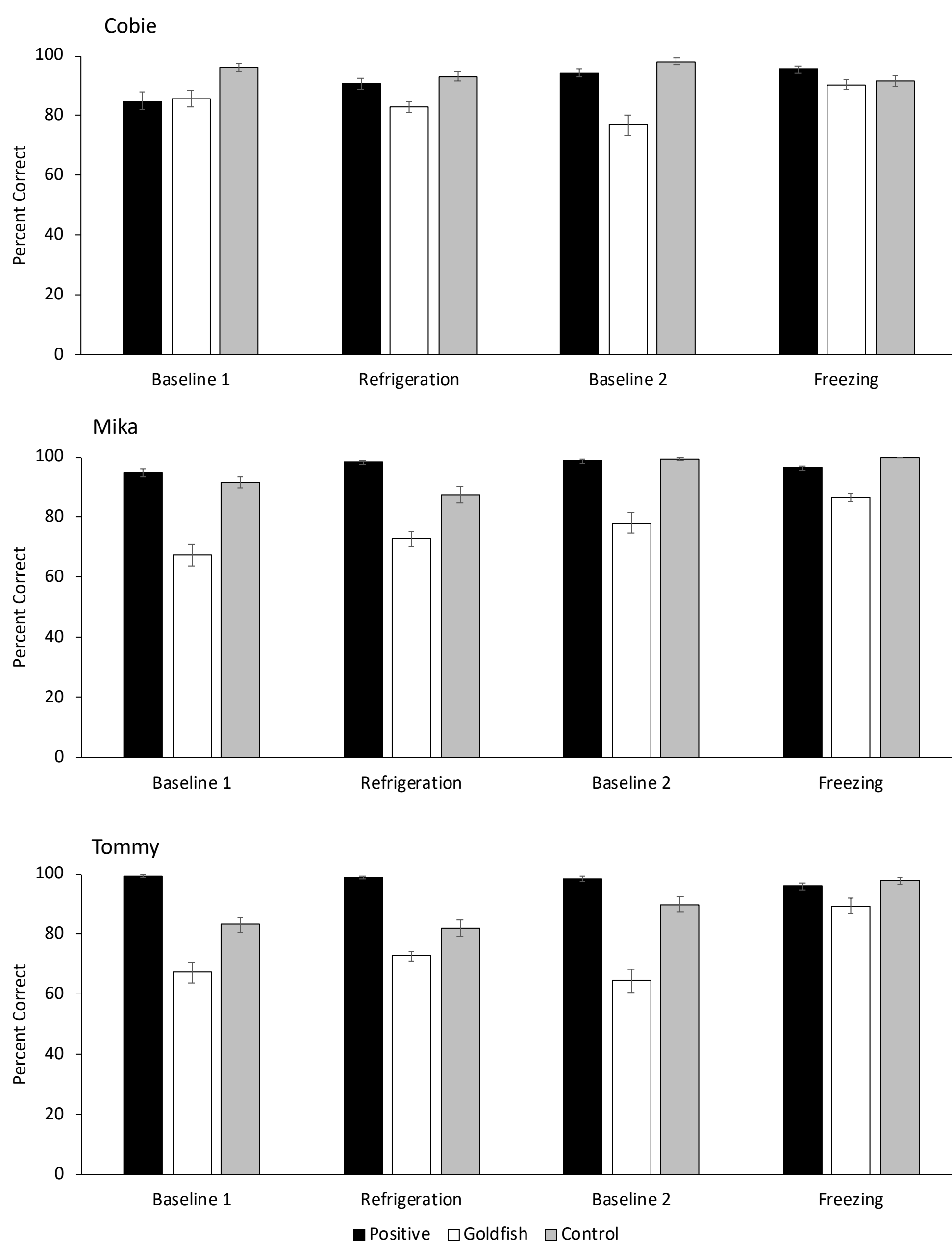


Table 3.1

Mean hit rate of positive samples, and mean correct rejection rates of control and goldfish samples during each phase of the preservation experiment

	Hit Rate				Control Correct Rejection Rate				Goldfish Correct Rejection Rate			
	Cobie	Mika	Tommy	Mean ± SE	Cobie	Mika	Tommy	Mean ± SE	Cobie	Mika	Tommy	Mean ± SE
Baseline 1	85%	95%	100%	93% ± 4.32	96%	92%	83%	90% ± 3.77	86%	68%	67%	73% ± 6.07
Refrigeration	93%	98%	99%	97% ± 1.98	93%	88%	82%	87% ± 3.33	83%	73%	73%	76% ± 3.41
Baseline 2	95%	99%	99%	98% ± 1.25	98%	99%	90%	96% ± 2.94	77%	78%	65%	73% ± 4.31
Freezing	96%	96%	96%	96% ± 0.26	92%	100%	98%	96% ± 2.49	90%	87%	89%	89% ± 1.12

3.3.3 d' calculations and ANOVA

The d' values are displayed in Table 3.2. All of the values are well above 2.0. The repeated measures ANOVA was conducted on Cobie, Mika, and Tommy's d' values to determine whether there was a statistically significant difference between the four phases of this experiment. The results showed that there was no statistically significant difference in the d' values between the baseline and preservation phases ($F(3, 6) = 2.061502$, $p = 0.2069$).

Table 3.2

d' values for the preservation experiment

	Cobie	Mika	Tommy
Baseline 1	2.37	2.44	3.28
Refrigeration	2.65	2.94	3.22
Baseline 2	2.80	3.43	3.04
Freezing	3.04	3.30	3.28

3.3.4 Accuracy relative to chance

Figure's 3.3 and 3.4 display the dogs' accuracy with 95% confidence intervals during each phase of the experiment and displays the accuracy that would be expected by chance. The 95% confidence intervals for each of the dogs at each of the phases do not cross over the calculated expected chance performance lines.

Figure 3.3

Cobie, Mika, and Tommy's hit rate (± 95% confidence interval) at each phase of the preservation experiment and the hit rate that would be expected by chance performance

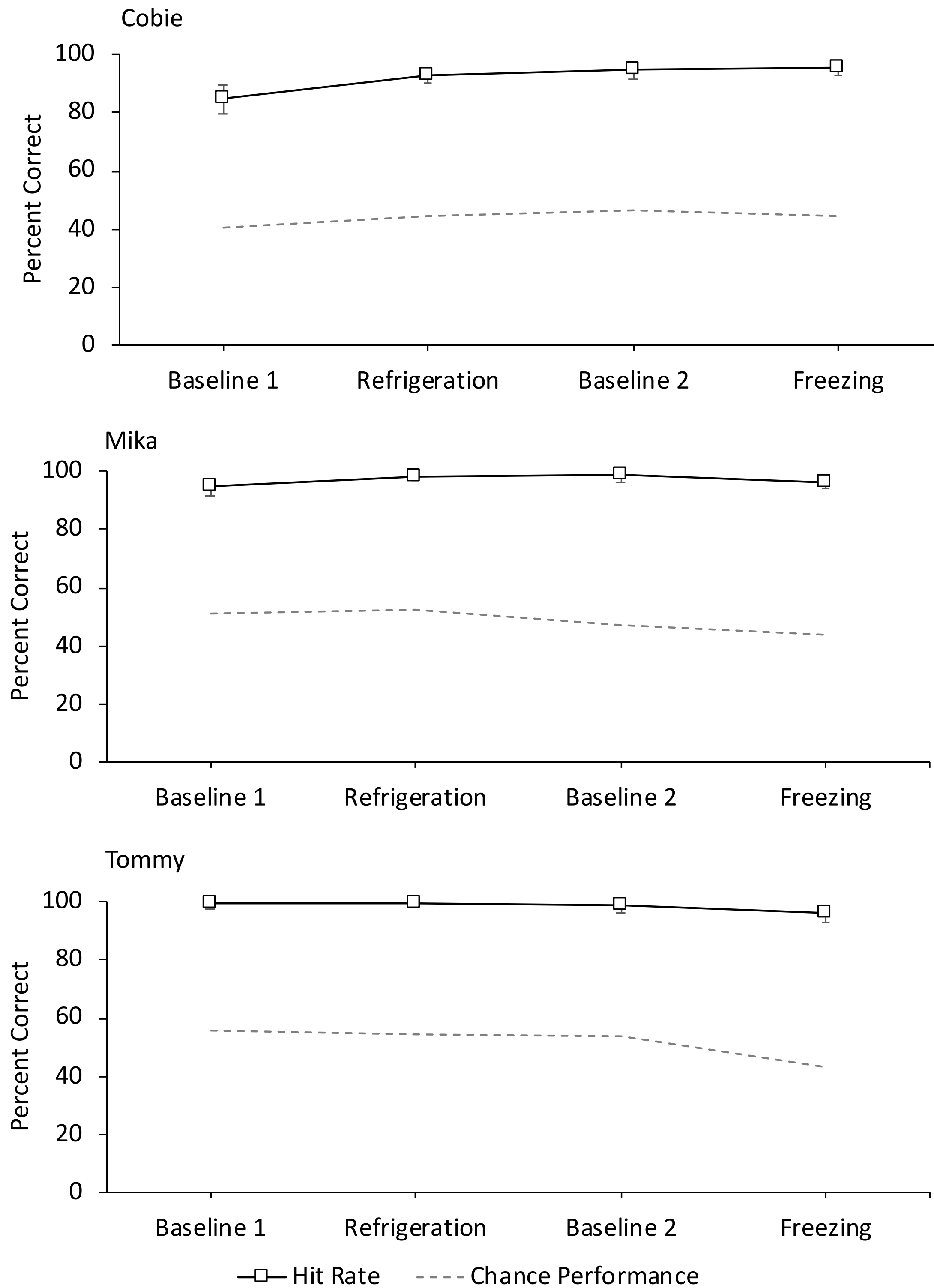
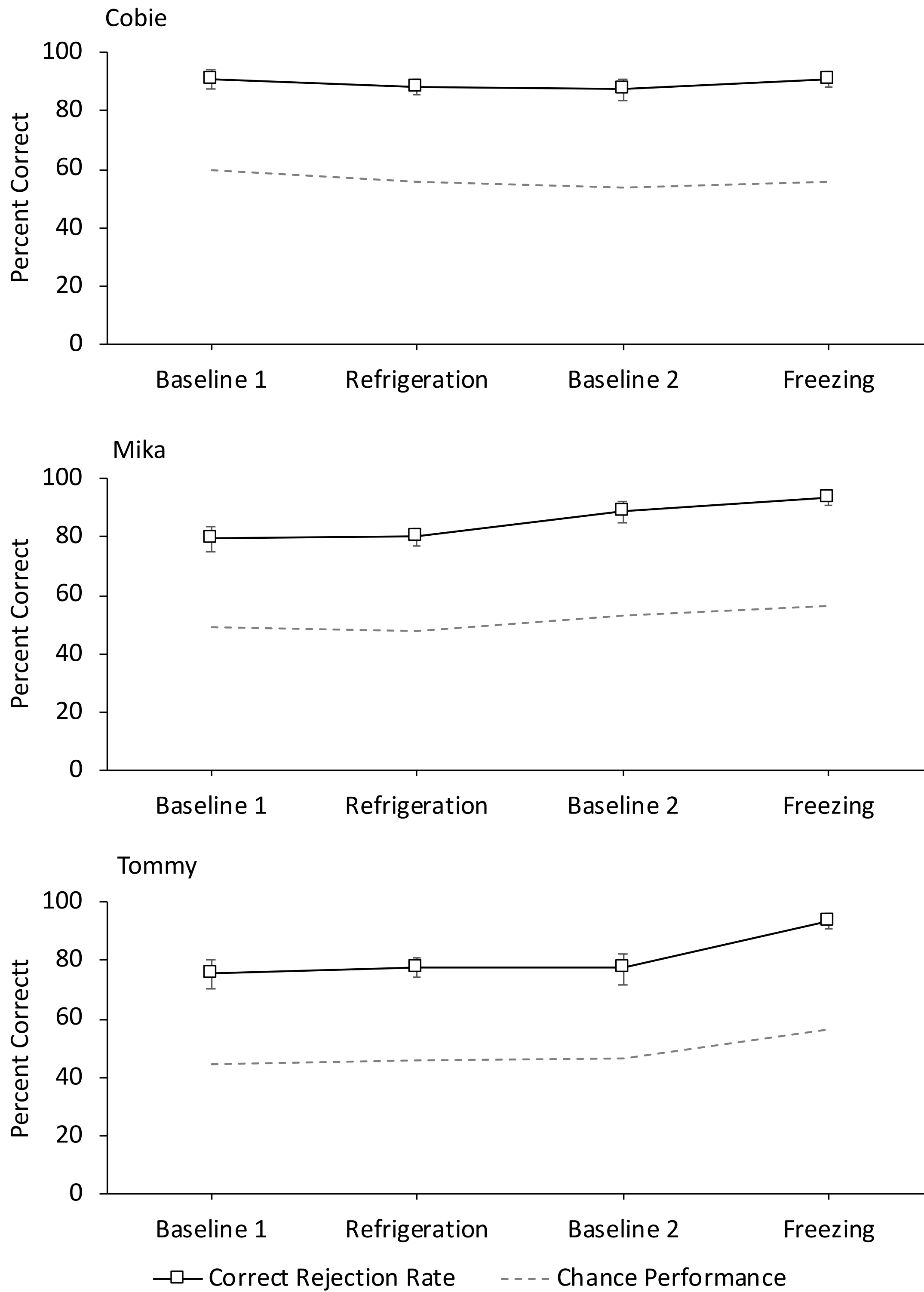


Figure 3.4

Cobie, Mika, and Tommy's correct rejection rate (± 95% confidence interval) at each phase of the preservation experiment and the correct rejection rate that would be expected by chance performance



3.4 Discussion

The objective of this study was to investigate the effect of preservation on the ability of scent detection dogs to detect water samples containing catfish odour and to discriminate against samples containing no-fish and goldfish odour. Water samples at a fish biomass concentration of 310 kg/ha were either refrigerated at 4°C or frozen at -20°C for approximately 1-week before being assessed by the dogs. The dogs maintained high correct rejection rates with their control samples throughout the phases, suggesting that their ability to detect fish was not significantly impaired by preservation. Goldfish correct rejection rates were lower, but this was consistent across the study phases, suggesting that preservation itself was not a factor in the reduced correct rejection rates. Additionally, the mean percent correct for each sample type for all dogs did not vary considerably between baseline (unpreserved) and preserved samples, and no statistically significant difference was found between the four phases of this experiment. All of the dogs had very high d' values for each of the phases which confirms their successful discrimination of signals from noise (Stanislaw & Todorov, 1999). This demonstrates that the preservation of samples did not influence the dogs' ability to detect and discriminate the target scent. Therefore, this all indicates that the refrigeration or freezing of samples could both be acceptable methods of preservation for catfish water samples collected from the field.

3.4.1 Refrigeration phase

The preservation of samples at 4°C did not have a significant effect on the dogs' ability to detect and discriminate catfish samples. This preservation phase had the advantage of the reversal back to baseline which allows us to see how the dogs' responding changes once the independent variable (refrigeration) is added as well as once it is removed (Johnston et al., 2020). The daily mean accuracy for all of the dogs' positive and control samples were high in the first baseline and remained high during the change to the refrigerated samples and back to non-preserved samples. The dogs' daily accuracy with the goldfish samples remained variable throughout all of these phases. This suggests that the move to refrigerated samples, and subsequent return to unpreserved samples, did not have a considerable effect on the dogs' ability to detect and discriminate catfish samples. This is important because it indicates that refrigeration could be a viable, practical method of preservation for samples collected from the field as the dogs were able to detect and discriminate them with similar accuracy to the unpreserved samples.

3.4.2 Freezing phase

The intended reversal back to baseline after the freezing phase was not able to be completed which means that we were unable to see how responding changed when the independent variable (freezing) was removed. However, the three phases prior to the freezing phase can help to provide insight into the changes, or lack thereof, in the dogs' accuracy observed in the freezing phase. As with the refrigeration and baseline phases, the dogs' accuracy with the positive and control samples remained high throughout the freezing phase. This again is important for the potential assessment of samples from the field that may necessitate freezing.

The dogs were not as consistent or as accurate with their goldfish samples throughout all phases of the experiment, but it is of interest that there were some observed improvements in the freezing phase. The mean goldfish correct rejection rates for all three dogs increased from 73% (± 4.31 SE) in baseline 2 to 89% (± 1.12 SE) in the freezing phase and visual assessment of the data appears to suggest more stability in the goldfish correct rejection rates in the freezing phase. Additionally, only Cobie was required to meet stability criteria before all of the participants progressed to the next phase, but all three dogs met the stability criteria in baseline 2 before being moved onto the frozen samples. Although there was not a statistically significant difference between the d' values of all four phases, it appears that the freezing of samples may have made it easier for the dogs to discriminate between the fish species. A reversal back to baseline would have helped to clarify whether the increase in accuracy for the goldfish samples was because the samples had been frozen and not because of an extraneous variable (Johnston et al., 2020).

The results from the refrigeration phase confirm that the refrigeration of samples could be a viable method to preserve field samples while maintaining hit rates and correct rejection rates. However, if freezing the samples has the added benefit of being able to increase the dogs' goldfish correct rejection rates and stability, it could suggest that for field samples, this preservation method be encouraged prior to assessment by the dogs. Further research that included a reversal baseline would be useful and the addition of frozen field samples would be critical.

3.4.3 Accuracy relative to chance performance

The dogs were all performing at levels considerably above chance again for this experiment. The dogs did not revert to indicating randomly once the samples had been preserved. It is crucial that they were able to do this as chance performance could not be tolerated when assessing real-world samples.

3.4.4 Concentration of samples

This preservation experiment used samples that were prepared at concentrations that are considered higher than would be biologically relevant for catfish because the dogs had previously been performing reliably at this concentration. Catfish are known to occur in Waikato lakes in densities of around 10-20 kg/ha (Tempero et al., 2019) which is considerably lower than the fish biomass concentration of 310 kg/ha used in this experiment. If the preservation experiment had been completed at these lower concentrations, the discrimination task may have been more difficult for the dogs, which would have made it challenging to determine whether any rejections of the samples were because of the preservation method or because of the low dilution. Therefore, it is suggested that further investigation into the preservation of samples at lower, biologically relevant concentrations be conducted.

3.4.5 Conclusions

The dogs were able to successfully maintain their accuracy when detecting and discriminating catfish samples which is important for the potential assessment of field samples that may be delayed in their assessment and thus require preservation. There was not a statistically significant difference across all four phases of the experiment but there was a noticeable increase in the goldfish correct rejection rates when the dogs moved to the frozen samples. High hit rates and control correct rejection rates were maintained throughout the experiment which suggests that water samples would be able to be either refrigerated or frozen before assessment by scent detection dogs.

Chapter 4
General Discussion

4.1 Real World Significance

4.1.1 Biomass Limits

The aim of this study was to investigate whether dogs could feasibly be employed to detect invasive catfish in aquatic biosecurity programmes in New Zealand. For this, scent detection dogs need to be capable of detecting their target species at concentrations at which they occur naturally in lakes, and all three dogs in this study demonstrated that this is possible. The dogs were all able to detect catfish at biomass concentrations <20 kg/ha which demonstrates their potential to be used in aquatic biosecurity programmes. Estimates of catfish biomass in lakes that were used for this study (i.e., 10-20 kg/ha) were obtained through netting and boat electrofishing mark-recapture efforts of established populations (Hicks et al., 2017; Tempero et al., 2019). Therefore, for scent detection dogs to be of use in monitoring programmes they would need to be able to detect new incursions of catfish when they are present at concentrations considerably lower than this, otherwise current detection methods would be sufficient. The fact that the dogs were successful at detecting catfish at very low concentrations, particularly Mika at 4.6 kg/ha, suggests that this could be achievable. Consequently, scent detection dogs could be employed in aquatic biosecurity monitoring programmes, as they have proven their ability to detect catfish at biomass concentrations lower than those currently on record for catfish concentrations in Waikato lakes.

All three dogs were able to detect catfish scent at <20 kg/ha (Cobie 18.6 kg/ha, Tommy 9.2 kg/ha, Mika 4.6 kg/ha), well within the expected biomass range for most catfish populations in the lower Waikato region (Hicks et al., 2017; Tempero et al., 2019). Although Cobie and Tommy did not reach the same end point in their dilutions as Mika, they may well have reached a similar dilution given more time as they only failed to meet criteria due to poor discrimination against the goldfish samples. The dogs demonstrated that they could discriminate between catfish, goldfish, and control samples not only at high concentrations (38,700 kg/ha) in the biomass limit experiment but also in the preservation experiment at 310 kg/ha. The lack of end point for Cobie and Tommy, and the fact that a lower concentration was not prepared for Mika,

means that we cannot know whether any of the dogs have reached a lower detection threshold. These results are also comparable to a study on common carp whose canine participant was able to detect common carp scent in water samples at 9.2 kg/ha (Collins, unpublished data), which was equivalent to Tommy's performance in this research, and surpassed by Mika's. This suggests that the biomass concentrations reached by the dogs in these studies are likely to be reached by other dogs that could be trained as invasive fish scent detectors.

4.1.2 Sample preservation

The practicality of sending and receiving samples to be assessed by a team of dogs would likely necessitate their preservation, especially if the sample collection location is remote or if the dogs are not available for immediate sample assessment. This study demonstrates that refrigeration or freezing of water samples for up to 1-week could potentially be used as viable methods to preserve real-world water samples before assessment by dogs. However, the observed increase in accuracy with which the dogs could correctly reject the goldfish samples in the freezing phase is of interest. There is limited published literature that examines the effects of sample preservation on dogs' scent detection abilities. However, a recent study that investigated the preservation of tall daisy samples had different results with the frozen samples than with the dried or fresh samples despite groups of dogs being trained on only either dried or frozen samples (Needs et al., 2021). The dogs were all successful at detecting the frozen, dried, and fresh samples but for all of the dogs, their first indication of a trial was most frequently a frozen sample suggesting that they had an increased ability to detect these samples over the others. The authors suggest that the frozen samples may have released more volatiles that allowed their scent to be picked up more readily by the dogs (Needs et al., 2021). A similar explanation could be true for the frozen samples in this current study and a chemical analysis of the volatile organic compound (VOC) profiles of the samples would be useful to understand any such changes. Gas chromatography-mass spectrometry (GC-MS) is a method of profiling VOCs that is used in various dog scent detection fields (e.g., Collins, 2018; DeGreeff & Peranich, 2021; Forbes et al., 2014). This process could be used for water samples like those in this current study to determine whether the VOC profile of the sample changes significantly between the preservation methods.

4.1.3 Comparison to current aquatic species detection methods

The catfish biomass concentrations that the dogs were able to detect are comparable to the detection capabilities of eDNA. In a recent study by Yates et al. (2021), the biomass density estimations of brook trout (*Salvelinus fontinalis*) from nine lakes ranged from 12.6 kg/ha to 52.5 kg/ha. These estimates were calculated from mark-recapture efforts involving a range of detection methods including fyke netting, angling, and backpack electrofishing (Yates et al., 2021). This indicates that at fish biomass concentrations of 12.6 kg/ha, eDNA can successfully detect the presence of fish. The present study found that scent-detection dogs can detect the presence of fish at concentrations lower than 12.6 kg/ha under laboratory conditions. This further supports the idea that scent detection dogs could be a valuable aquatic species detection tool as they could be more sensitive than current methods, and surveys could occur more frequently. Further research is needed to determine if similar detection levels can be reached in real world conditions.

Similar to eDNA, scent detection dogs could be useful to detect species that are more difficult to detect through traditional methods due to their cryptic nature and low abundances as no individual organisms have to be present for their detection (Bohmann et al., 2014; Jerde et al., 2011). In comparison to the more traditional methods, both eDNA and scent detection dogs are both comparatively time and cost effective (Itakura et al., 2019; Jerde et al., 2011). In addition, many of their disadvantages are shared such as that no specimens are caught to gain further knowledge about individuals, and crucially, there is the potential for false positives (Bohmann et al., 2014; Clusa & García-Vázquez, 2018; Trebitz et al., 2017). If a sample returned a positive result from either eDNA or scent detection dogs, manual methods would still need to be applied to remove the individuals in invasive species management. This could result in a waste of resources if it was a false positive. However, dogs can be more sensitive than instruments and manual detection methods in some circumstances (Cablk et al., 2008; Helton, 2009), which means that if a dog indicated a lake to be positive and no individuals were found, it does not necessarily mean that the dog was incorrect. eDNA has an initial high cost in terms of developing species-specific amplification primers (Trebitz et al., 2017) and scent detection dogs can be expensive to train (Gsell et al., 2010; Long et al., 2007). With many of these factors shared among the detection methods, the fact that the dogs in this current study could detect catfish at biomass concentrations lower than what is currently recorded for eDNA is important for their potential future employment in biosecurity programmes.

4.1.4 Advantages of laboratory over field

Theoretically, a dog or a team of dogs, could be taken out to a lake and asked to indicate whether the water contained catfish as has occurred with wastewater contamination detection dogs in the United States (Van De Werfhorst et al., 2014). This is advantageous for wastewater and other point discharges but this is not practicable for detection of diffuse contaminants or invasive species (Yick et al., 2021).

One advantage of a laboratory-based approach to invasive species detection is the use of the self-operating apparatus. With this apparatus, the dogs work completely on their own which means that any potential for cueing by a dog handler is entirely eliminated and we can be confident that the dogs are working through their own volition (Edwards, 2019). Many scent detection studies state that by having a double-blind approach, they have ensured there is no unconscious cueing by the handler as they do not know the location of the target sample and therefore cannot indicate any information to the dog (DeShon et al., 2016; Rutter et al., 2021; Van De Werfhorst et al., 2014). However, as demonstrated by Lit et al. (2011) dogs can cue off their handler even when the handler is unaware that there is no target scent present and therefore believes that there should be. Therefore, the effectiveness of genuinely eliminating cueing achieved by the self-operating apparatus in this study cannot be overstated.

If in the field, dogs would have only one sample to assess, the lake. In the laboratory, dogs can assess many samples from many lakes, and there can be control samples present to help with assessment of the dogs' performance. Additionally, not knowing the status of catfish in a lake in the field would mean that the dogs could not be reinforced for a correct indication. With the laboratory-based apparatus, not only can we present to the dogs unknown samples from a lake for which the dogs would not receive reinforcement for indicating on, but we can have known positive samples for which the dogs' indications can be reinforced to help with their performance and motivation.

Another advantage to a laboratory setting is that the environmental conditions in the room can be controlled, which includes the temperature but could also be developed to include humidity. Studies have demonstrated that explosives detector dogs' abilities are affected by humidity

(McLean et al., 2005); thus a control for this could be useful. There is also no wind or noise from other outdoor smells that would occur in the field and in a purpose-built facility, the option for a work room away from other distractions such as people coming and going, and cars driving by would be beneficial.

4.2 Limitations

4.2.1 Use of volunteer pet dogs

Dogs can be extraordinary assets in scent detection work (e.g., Becker et al., 2017; Bellingham et al., 2010; Rolland et al., 2006), but there are still a lot of aspects that cannot be controlled for despite our best efforts. The use of pet dogs in this research meant no control was able to be had over anything that the dogs did outside of the laboratory including, how much they ate, what they ate, and how often they were exercised. On occasion, this had an effect on how they performed. The only practical way to eliminate this would be to have trained working dogs in place of volunteers which would mean that their diet, exercise, and out-of-work circumstances could be controlled, and they could work more reliably. However, the dogs in this study worked through their sessions each day, and they had great accuracy which provides confidence that even though they were volunteers, the data that these dogs produced is still of excellent quality.

4.2.2 Motivation of the dogs

All three dogs that took part in the experiments experienced motivation issues at one time or another. One of which, with Mika and Tommy, was mitigated with the change in reinforcer from kibble to Possyum. Another matter for consideration when working with dogs is that they can become fatigued or bored (Dahlgren et al., 2012; DeShon et al., 2016). This may have been an issue in the biomass limit experiment, which had longer sessions, as the dogs often took longer to complete their last session of the day than their first three or did not complete it at all. Once the sessions were shortened for the preservation experiment, the dogs worked through all of their expected number of sessions. The dogs were also only ever working for less than an hour each day, usually between 35-45 minutes inclusive of rest time. Therefore, these dogs were not working for nearly as long as dogs in many other studies (Becker et al., 2017; Matthew et al., 2021; Wasser et al., 2004). Although these obstacles occasionally stalled the smooth running of the experiments and meant that data collection for some of the dogs was halted, the

potential for many of these issues to arise could never be completely eliminated when working with dogs whether they are highly trained working dogs or volunteers (Dahlgren et al., 2012; DeShon et al., 2016). Also, all complications with the three dogs that participated in this study were successfully resolved and they were able to produce sufficient data.

4.2.3 Standard dilution in biomass limit experiment

The 38,700 kg/ha standard dilution that the dogs worked at during the biomass limit experiment was very high and considering their later success in the preservation experiment at 310 kg/ha, the dogs could easily have worked at a lower dilution. However, the reason that 38,700 kg/ha was chosen was because that is where the previous student working with these dogs ended her experiment. It would have been more useful if the dogs worked at a lower standard dilution, but there was limited time within which to complete the experiment. In hindsight, the standard dilutions could have been lowered before both the biomass limit and preservation experiments commenced because the dogs achieved that very quickly in the transitional stage between the two experiments. Regardless of the standard sample solution being so high, the dogs could still detect the probe samples and discriminate them from the others.

4.3 Future research

The natural next stage of this investigation would be to have dogs assess samples that are prepared using real lake water as this would establish whether scent detection dogs could be employed in practical biosecurity situations. Samples from lakes usually have more noise in them from scents belonging to other aquatic species as well as contaminants from runoff, likely making it more difficult for the dogs to discriminate between samples with and without catfish. The first step would be to use lake water that has no catfish present in the same way that the control aquarium water was used in this study i.e., it would have known concentrations of catfish and goldfish (or another distractor scent) added to it for the dogs to assess. The next step would be to prepare samples from lake water that is known to be contaminated with catfish and other samples from another lake that is known to be free of catfish. If successful in discriminating which of the samples are from lakes contaminated with catfish from those that are free of catfish, this would be further evidence that scent detection dogs could be used for this kind of work. Research investigating dogs' ability to detect common carp in real-world water samples is currently being carried out with great success (Collins, unpublished data).

If scent detection dogs were employed to detect invasive fish species in water samples from lakes, it is likely that said samples would have to be preserved for practical reasons before they are assessed by dogs. Thus, the effects of preservation on real-world samples would need to be investigated similarly to how they were in this current study. Additionally, research into the effects of the length of time samples are preserved would be beneficial as it is likely that these times would vary. It would be important to know whether too much or too little time preserved would be detrimental to the samples or the dogs' detection abilities.

A final suggestion for future research, or even for maintaining training, would be to randomly introduce the dogs to novel distractor scents. When assessing real world samples, the dogs will come into contact with many more scents than they could ever be trained with in the laboratory, therefore it could prove useful to change the distractor scent from time to time (Oldenburg et al., 2016). This helps maintain the dogs' generality and ensures that they know that the catfish scent is what they are looking for rather than that goldfish, for example, is what they are not looking for.

4.4 Conclusions

Scent detection dogs have the potential to be employed in biosecurity programmes detecting the presence of invasive species in water samples. They can successfully detect catfish at concentrations that would naturally occur at in lakes levels that are comparable to other survey methods. The dogs' ability to detect and discriminate catfish in preserved samples is advantageous as, if the dogs were to be used operationally in this manner, the majority of samples from lakes would likely necessitate such preservation. Their catfish detection success coupled with the fact that the method by which they assess samples is comparatively low-cost, quick, and straightforward means that there is a real possibility that this could one day be a viable detection method. Use of scent detection dogs could facilitate more regular monitoring of lakes as it is far less strenuous than the methods that are currently in place. More research is needed to be conducted to confirm the dogs' ability to achieve similar excellent results with real-world water samples.

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Appendices

Appendix A

Standard Operating Procedure - Training Dogs for Scent Detection Work Using Automated Apparatus

Note: This procedure does not include dog selection, habituation, handling, and care as requirements are likely to vary among laboratories. The complete standard operating procedures specific to the author's laboratory are available on request.

Apparatus Setup

Position the apparatus in a room without other objects that might distract the dog. Only the front panel should be accessible to the dog, a ramp may be required so the dog can access the sample port hole. Movable partitions may be used to block access to the other sides of the apparatus. The room must have a door that closes/latches and should be equipped with one or two cameras to monitor the dog. The computer(s) used to control the apparatus and monitor the dog should be positioned in an adjacent room.

Basic Training

1. **Introduction**

   Once the dog has been habituated to the environment and the researcher(s), training sessions can be started. During the shaping and early training process, at the first sign of fatigue to disinterest, the session should be terminated, ideally immediately following a correct response and reinforcement. Early shaping/training sessions should not exceed 5 minutes. Dogs should be given a short break between sessions.

2. **Conditioned Reinforcer Establishment**

   The researcher should enter the experimental room with the dog and stand to the side of the apparatus (the side closest to the door is preferred if possible). The researchers should stand with their hands crossed either in front of their body or behind their back

(whichever is more comfortable), holding the feeder remote/hand-switch out of view of the dog. The dog should be allowed to freely explore the experimental room. Dispense food from the automatic feeder using the remote/hand-switch until the dog immediately approaches the feeder upon hearing the sound made when the feeder is activated. Take care not to trigger the feeder if the dog is only sitting and staring at the feeder. The dog should approach the automatic feeder and consume the food within 3 seconds of activation 3 times in a row to continue to the next stage of training.

3. **Shaping- Nose to Port**

Once the sound of the feeder is established as a conditioned reinforcer, the remote/hand switch is used to train the dog to put its nose into the sample port of the apparatus. Use the method of differential reinforcement of successive approximations to target this behaviour. For initial sessions the apparatus should be turned off, and not loaded with samples but one segment may be placed on the apparatus for the dog to open. The closing of a segment does make a sharp tap noise which can sometimes initially startle the dog. Prompting (e.g. pointing) may be used, but the prompt must be faded and removed before processing to the next step (lever activation).

As soon as the dog is comfortably placing its nose into the port far enough to open the segment and make the closing noise, the dog should be removed from the room. A single, positive sample is placed at position one of the apparatus. The participant's configuration file on the computer should then be edited to set the response times as 1000ms for the minimum indication time and 500ms for observations. The apparatus will now make a beep sound when the dog places its nose in the port. Continue shaping as required until the dog begins to trigger the feeder automatically. Once a run (17 samples) at the 1000ms threshold is complete, increase the threshold in 100-500ms intervals to 2500ms. Once a run is complete at 2500ms, continue to the next step.

4. **Shaping – Omni-directional Switch Activation**

With the apparatus unloaded and turned off, use the method of differential reinforcement of successive approximations to shape lever pressing. Depending on the size and behavioural tendencies of the specific dog, an appropriate topography should

be selected for shaping (e.g., use of a paw or nose to activate the lever/omnidirectional switch). Prompting (e.g., pointing) may be used, but the prompt must be faded and removed before processing to the next step. Once the lever has been activated 10 times without prompts (and reinforced via manual activation of the feeder), proceed to the next step.

5. **Discrimination Training**

   A positive sample is placed at position one on the apparatus and a control sample is placed at position two. A full set of 17 samples is not required for training. The participants configuration file should be updated to alternate between the positive and control sample. The samples contain 100% of their target scent water.

   Bring the dog into the experiment room and stand in place beside the apparatus. If there is no response given to the apparatus within 20 seconds, prompt as required. When the dog encounters the first negative sample, allow 10 seconds before prompting to see if lever pressing occurs without prompt. Continue prompting when necessary, but fade out prompts as soon as possible (e.g., wait for increasing amounts of time before prompting). Be sure to prompt with a consistent cue.

   Once one run has been completed without prompting, randomise the order that the samples are presented to the dogs in the configuration file. The same randomisation pattern may be used for up to a maximum of 4 carousel rotations in a row before it is needed to be randomised again. Continue until hit rate (correct positive indication) and rejection rate (correct lever pressing) are above 80% without prompts.

   At this point the experimenter should gradually remove themselves from the room and, once the dog is successfully working on their own, systematically increase the indication threshold in 100-500ms increments until they reach the target threshold (5500ms is generally optimal based on our preliminary research, but this may vary depending on the dog/application).

Advanced Training

1. Introducing New Samples

To introduce a new sample that is to be treated like a negative, first you must systematically increase the number of negative control samples and decrease the number of positive samples (e.g. if you are going to be introducing 5 new samples then you need to increase the number of negative samples to 10 and decrease the number of positive samples to 7). If the dog is still performing well, swap the corresponding number of negative control samples with the number of new negative non-target samples.

It may be necessary to re-enter the room and provide prompts, but then the experimenter must be sure to phase out prompting as soon as possible and gradually remove themselves from the room again.

2. Decreasing Sample Concentration

Once the dog is reliably performing above 80% correct hit and rejection rates after the introduction of new samples, it is possible to start to decrease the sample concentration (Table 1). Dilutions should be done incrementally and for both target (positive) and non-target (negative) samples, the criteria for going down a dilution is a correct hit and rejection rate above 80% for two out of three sessions.

Table 1

Dilution	Volume Control (ml)	Volume Target (ml)
1st Dilution	50	50
2nd Dilution	75	25
3rd Dilution	87.5	12.5
4th Dilution	93.75	6.25

Trouble Shooting Tips:

If the dog is performing poorly in training:

- Make sure the dog is healthy, deal with any health-related issues first.
- Confirm the dog is not being fed by the owner at least 2 hours prior to training.
- Confirm that there has been no significant changes in the dogs home routine (e.g. owner has been away for an extended period, new dog introduced at home, change in diet, fireworks have been let off recently etc.)
- Confirm that food is an effective reinforcer by evaluating approach and consumption and/or by attempting to shape a simple response. If confirmed try selecting a different food (using paired-choice preference assessment procedure).
- Check factors related to sample quality (make sure that samples have been prepared and arranged as specified in the specific sample preparation SOP).
- Return to earlier stages of training as required (e.g. if the lever press is not occurring reliably in discrimination training conduct another lever press shaping session in isolation).
- If dog continues to perform poorly consult with supervisor. The dog may need to cease participation in the study.

If the dog is putting their nose in the port too early (while the apparatus is still moving):

- Turn on the "noise mode" in the participants configuration file. The apparatus will now produce a "buzz" while the carousel is still moving.
- Use a board to create an obstacle the dog must navigate around in order to reach the lever/omnidirectional switch and return to the port.

Guide for Shaping

1. **Introduction**

This outlines the basic training hierarchy for shaping by successive approximations. As a general rule, each step must be completed three times in a row before progressing to the next stage of training. Some dogs, however, may require additional learning trials before progressing. Keep sessions short (under five minutes) and finish on a positive note when possible, to ensure that the process is enjoyable for the dog.

2. Procedure

Researcher is to position themselves near the apparatus, ideally near the door, avoiding the dog's gaze to reduce unintentional cueing. This will facilitate fading of the researcher's presence during later trials when the dog is required to be in the experimental room alone. Gestural prompts may be used to facilitate training, but these should be used only as needed as they must be faded out before training is complete.

2.1 Shaping of sample port entry

2.1.1 For initial sessions the apparatus should be turned off, and not loaded with samples but one segment may be placed on the apparatus for the dog to open (The closing of a segment does make a sharp tap noise which can sometimes initially startle the dog).

2.1.2 Reinforce moving further and further away from the feeder, until the dog is reliably approaching the side of the room near the apparatus.

2.1.3 Reinforce attending to the apparatus (putting nose near or on any part of the front panel).

2.1.4 Reinforce nose near port.

2.1.5 Reinforce nose in port.

2.1.6 Reinforce nose touching and opening the flap (indicated by a tap noise as it closes).

2.1.7 Reinforce pushing flap inwards.

2.1.8 Turn the apparatus on – when the sample port beam is broken it will now produce a "beep" sound.

2.1.9 Continue to reinforce for dog breaking the beam and pushing the flap inward, until the dog is fully opening the flap (nose is fully inside the port).

2.2 Shaping of lever press

2.2.1 Turn apparatus off. Do not have apparatus loaded with samples.

2.2.2 Reinforce any movement towards the lever.

2.2.3 Reinforce movement of nose or paw toward the lever (as appropriate).

2.2.4 Reinforce any contact with the lever (nose or paw, as appropriate).

2.2.5 Reinforce any movement of the lever.

2.2.6 Reinforce movement of the lever that produces a "click" (microswitch closure).

Appendix B

Standard Operating Procedure – Acid Washing for Fish Projects

1. Purpose

This standard operating procedure provides guidelines and standardised procedures to be adopted during the acid washing of glassware used during scent detection (fish) projects in the R.2.38 laboratory on the University of Waikato Hamilton campus. Only those with prior induction training are authorised to do this.

2. General Rules

2.1. Laboratory rules should be followed at all times.

2.2. Two people must be present to do nitric acid washing.

2.3. Acid washing may not be completed after hours (after 5pm), without prior approval.

2.4. Glassware is to be left in acid overnight.

3. Putting Into Acid

3.1. Hydrochloric acid washing – Sample bottles only

3.1.1. A lab coat, safety glasses and disposable gloves should be worn.

3.1.2. There are acid buckets of 10% HCl designated to each sample type, this is labelled on each bucket. There are also labelled long, green gloves designated to each sample type/bucket.

3.1.3. Bottles should be put in their designated buckets in order from negative to positive sample type.

3.1.4. Sample bottles should have all been emptied down at the dog lab (TT.H.4).

3.1.5. Wear the designated long green gloves.

3.1.6. Remove the lid from the bucket.

3.1.7. Remove the lid from the sample bottles to be placed in the bucket.

3.1.8. Place the bottle into the acid solution, do so at a slight angle so the bottle can fill with acid but does not bubble violently. Ensure there are no air bubbles in the bottle. Bottles should be fully submerged.

3.1.9. Place the bottle lids into the acid solution, be sure to submerge them.

3.1.10. Replace the bucket lid.

3.1.11. Rinse the green gloves with reverse osmosis (RO) water and return them to the correct space beside their designated bucket.

3.1.12. Repeat steps 3.1.5 to 3.1.11 for each sample bottle type.

3.2. Nitric acid washing – All other glassware

3.2.1. All glassware used other than the sample bottles must be washed in nitric acid.

3.2.2. A lab coat, safety glasses, disposable gloves, long green gloves, a protective apron, and full face mask must be worn when interacting with the acid.

3.2.3. Empty glassware to be cleaned (cylinders, funnels, beakers and sample jars) onto plastic tray next to the fume hood.

3.2.4. Put on the pair of long green gloves in the fume hood. Check the gloves thoroughly for any cracking, if gloves are deteriorated replace them with new ones.

3.2.5. Remove the glass lid from the acid bath.

3.2.6. Using tongs (located in a container in the fume hood), carefully place the glassware into the acid bath. Ensure there are no trapped air bubbles and the glassware is fully submerged. Cylinders can only be placed in the large acid bath on the left hand side as it is the only bath deep enough to be able to submerge properly.

3.2.7. Replace the acid bath lid.

3.2.8. Rinse the tongs and long green gloves thoroughly in the sink, and return them to the fume hood. Be sure to make sure you've properly closed the fume hood.

3.2.9. Remove protective apron and face mask.

3.2.10. Dispose of gloves appropriately.

4. Taking Out Of Acid

4.1. Hydrochloric acid – Sample bottles only

4.1.1. A lab coat, safety glasses and disposable gloves must be worn.

4.1.2. There are acid buckets of 10% HCl designated to each sample type, this is labelled on each bucket. There are also labelled long green gloves (need appropriate names for these) designated to each sample type/bucket.

4.1.3. Bottles should be handled in order from negative to positive sample type.

4.1.4. Wear the designated long green gloves.

4.1.5. Remove the lid from the bucket.

4.1.6. Remove the sample bottles from the bucket, tip the acid out carefully and slowly to avoid splashing.

4.1.7. Place the bottle into the labelled designated rinsing bucket.

4.1.8. Remove the lids from the acid solution and place these into the labelled designated rinsing bucket.

4.1.9. Replace the acid bucket lid.

4.1.10. Rinse the bottles and lids in RO water, and place them on the rinsing bucket lid for transport to the drying incubator.

4.1.11. Rinse the long green gloves with RO water and return them to the correct space beside their designated bucket.

4.1.12. Take the rinsed bottled to the incubator for drying. Control bottles should be placed on the top shelf, negatives on the middle shelf and positives on the bottom shelf.

4.1.13. Dispose of and change gloves (if applicable).

4.1.14. Repeat steps 4.1.4 to 4.1.13 for each sample bottle type.

4.1.15. Leave glassware in incubator to dry overnight.

4.1.16. Wearing gloves put clean dry glassware in designated storage containers.

4.2. Nitric acid – All other glassware

4.2.1. A lab coat, safety glasses, disposable gloves, long green gloves, a protective apron, and full face mask must be worn when interacting with the acid.

4.2.2. Ensure the rinsing buckets are full with RO water prior to interacting with acid.

4.2.3. Put on the pair on long green gloves in the fume hood. Check the gloves thoroughly for any cracking, if gloves are deteriorated replace them with new ones.

4.2.4. Remove the glass lid from the acid bath.

4.2.5. Using tongs (located in a container in the fume hood), carefully remove the glassware from the acid bath. Empty glassware of acid as much as possible, and then submerge in the rinsing bucket.

4.2.6. Replace the acid bath lid.

4.2.7. Rinse the tongs and long green gloves thoroughly in the sink, and return them to the fume hood. Be sure to make sure you've properly closed the fume hood.

4.2.8. Remove protective apron and face mask.

4.2.9. Dispose of gloves and replace.

4.2.10. Once the glassware has been submerged in the RO water for at least 10 minutes, rinse with RO water and place onto plastic container to transport to the incubator.

4.2.11. Place glassware in incubator.

4.2.12. Dispose of gloves.

4.2.13. Leave glassware in incubator overnight to dry.

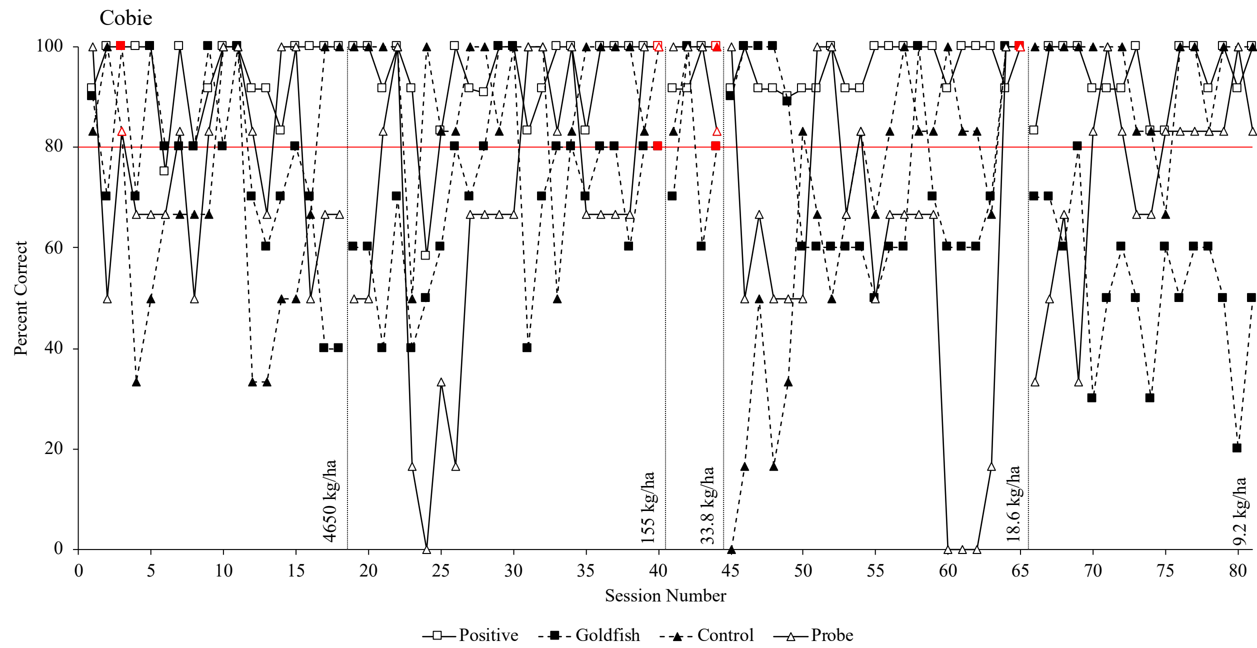
4.2.14. Wearing gloves put clean dry glassware in designated storage containers.

Appendix C

Session-by-session Data for Biomass Limit Experiment

Figure 1

Cobie's session-by-session data for the biomass dilution experiment

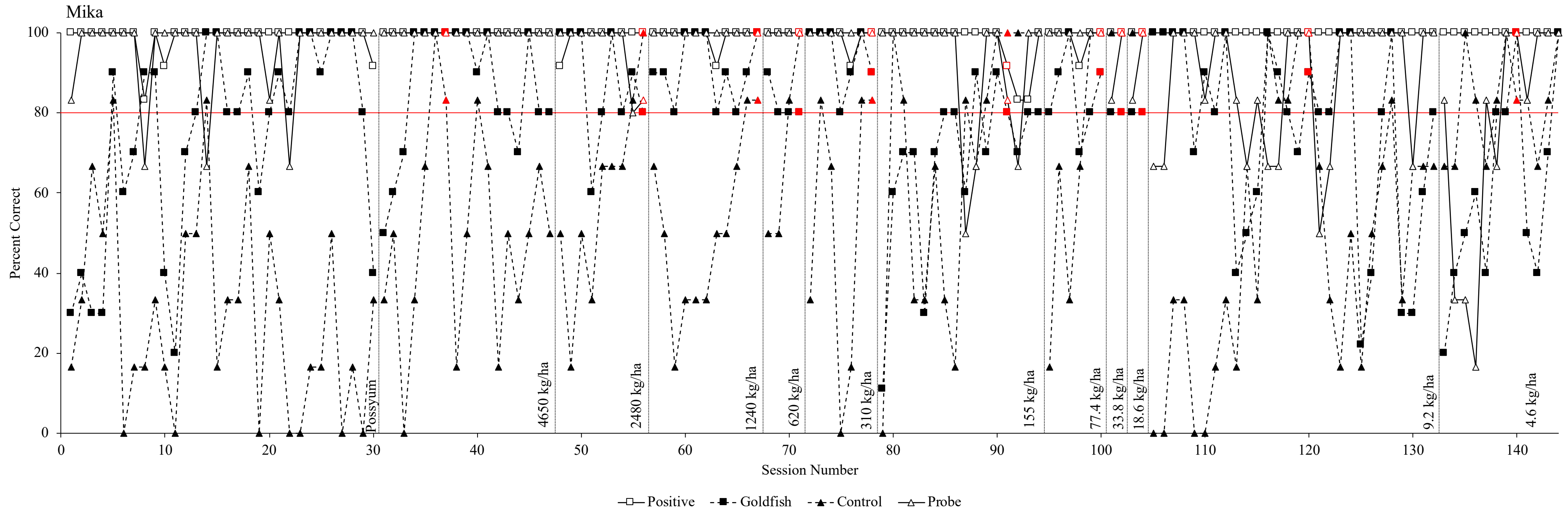


Red line at 80 percent correct indicates threshold to meet criteria.

Red markers indicate the first session that the dog met criteria at that dilution.

Figure 2

Mika's session-by-session data for the biomass dilution experiment

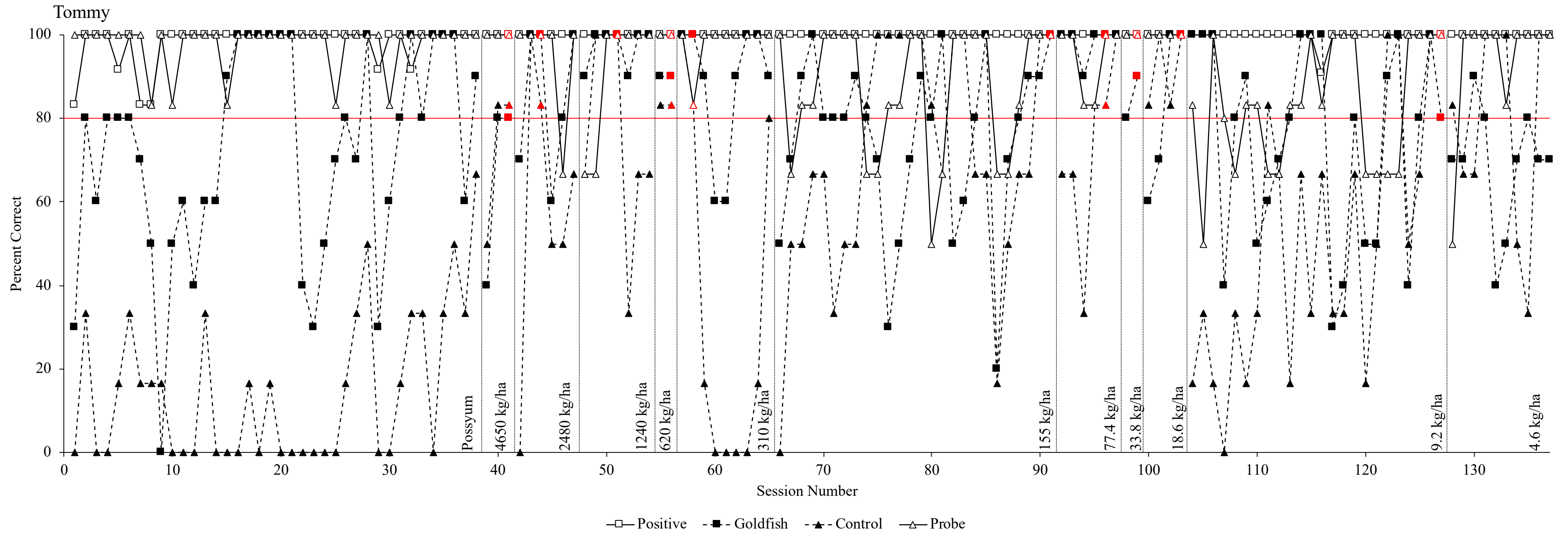


Red line at 80 percent correct indicates threshold to meet criteria.

Red markers indicate the first session that the dog met criteria at that dilution.

Figure 3

Tommy's session-by-session data for the biomass dilution experiment



Red line at 80 percent correct indicates threshold to meet criteria.

Red markers indicate the first session that the dog met criteria at that dilution.

Appendix D

Session-by-session Data for Preservation Experiment

Figure 1

Cobie's session-by-session data for the preservation experiment

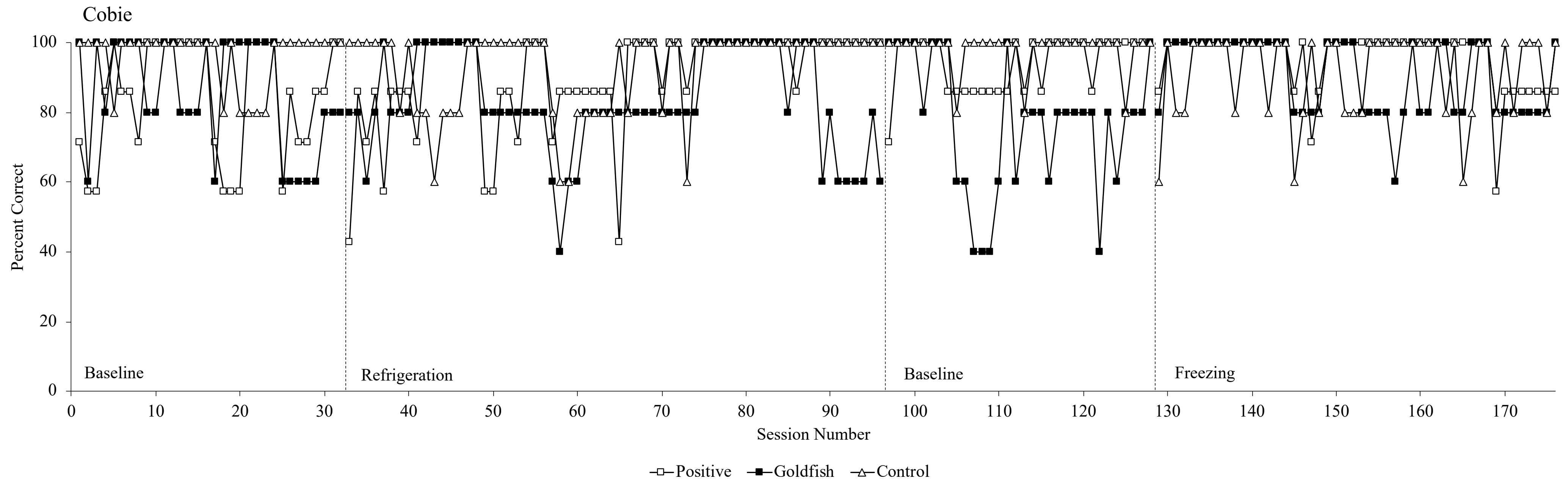


Figure 2

Mika's session-by-session data for the preservation experiment

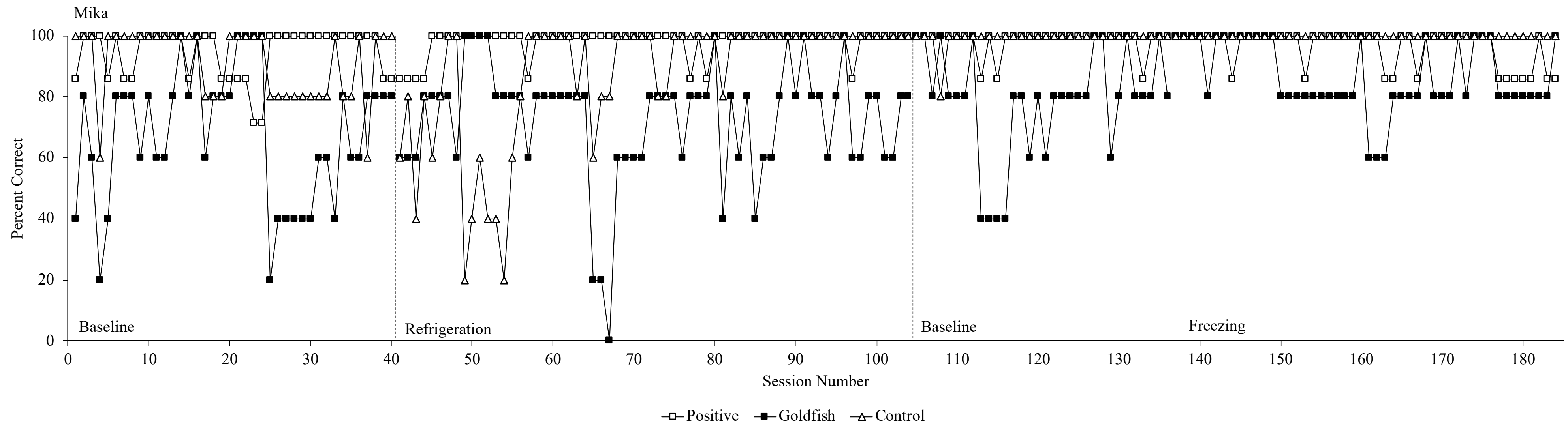


Figure 3

Tommy's session-by-session data for the preservation experiment